The Gypsy Moth: Research Toward Integrated Pest Management

Edited by
Charles C. Doane
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Foreword

In 1973 two chronic forest insect problems, the gypsy moth in the Northeast and the southern pine beetle in the South, were severe. The tussock moth outbreak in the Pacific Northwest was climaxing that year as well. The extensive damage caused by these three insects caused national concern in the private as well as in the public sector. In August 1973 the Assistant Secretary for Conservation, Research, and Education, U.S. Department of Agriculture, requested that four agricultural agencies—the Agricultural Research Service, the Animal and Plant Health Inspection Service, the Cooperative State Research Service, and the Forest Service—develop coordinated short-term programs to reduce damage caused by the three pests. The Congress provided the necessary funds for the Combined Forest Pest Research and Development Program in a special appropriation bill signed by the President in August 1974. A program board, the members of which were the heads of these four agencies and four knowlegeable administrators from research and user groups, participated in the planning and reviewed annual plans of work and budgets. Overall coordination of the three-pest program was provided by the Office of the Secretary.

Transfer of technology resulting from the three CFPP programs was of major concern to this office. Program managers were directed to plan for the most effective means of getting knowledge gained in the program to the planners and managers who needed it. One of several methods chosen was to assemble all known information on the insect into a single source—this book.

The Gypsy Moth: Research Toward Integrated Pest Management is the effort of many scientists from the Department of Agriculture, universities, and State agencies. Although it does not contain all the answers to the gypsy moth problem, it does contain new or improved methods for control. Equally important, this work defines continuing research and development needs essential to improve further the methods of coping with this destructive insect. The quality and amount of sound and useful information presented in this compendium demonstrate the value of cooperative research by Federal, State, and university scientists and practitioners representing a variety of disciplines and experience. Such research must continue, however, if we are ultimately to provide effective protection to our forest resources through fully integrated pest management systems.

M. Rupert Cutler
Assistant Secretary for Natural
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Contents

Acknowledgments

Many individuals contributed to the preparation and publication of this book. Although authors are appropriately cited throughout the text, the manuscripts reflect only a small number of the total scientific work force that conducted research during the 1975–78 expanded program. Unrecorded by name in many instances are the technicians, administrative support staffs, students, graduate students, and postdoctoral fellows who played a major role in completing research within the constraints of time. The efforts of these individual scientists have made it possible to report here the many accomplishments attained during the expanded research and development program.

The incentive for sustaining high-quality research and maintaining the continuing sense of urgency to complete assignments had full support of the Department of Agriculture's Office of the Secretary and affiliated agency heads and staffs. The continual support and participation of university, State agriculture experiment station, and industry specialists were also essential to the total scientific effort.

Publication of a major treatise requires much coordination and effort. The end product appears after hours of tedious work and support of publication specialists. Recognition is therefore appropriate to those who have made publication of this book possible. First, to the staff of the Office of Governmental and Public Affairs, Department of Agriculture, who worked diligently and efficiently on every phase of this publication, greatest appreciation is extended for their dedication, constructive criticism, and productivity throughout the entire process. Dr. Michael McManus, Program Research Coordinator, and Dr. Charles Doane merit gratitude for planning the publication and for serving as technical editors. To Mary Sommerville, sincere thanks is expressed for her dedicated, enthusiastic role as compendium editor and publications coordinator.

We trust you will find the pages that follow enlightening, scholarly, and of value as a result of the efforts of all these people.

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Introduction

Michael L. McManus and Thomas McIntyre

The Problem

The gypsy moth, *Lymantria dispar* L., is now well established as a serious defoliator of forest, shade, and fruit trees and ornamentals over much of the Northeastern United States. The gypsy moth is the only forest insect under regulation of a U.S. Department of Agriculture Federal Domestic quarantine. Since its accidental introduction into this country from Europe in the late 1860's, this insect has received singular attention from research, regulatory, and control specialists of many disciplines. Possibly no other forest insect has been studied as thoroughly or has been the target of such intense containment, control, or eradication strategies.

Despite all efforts, the gypsy moth has persisted and continues to extend its range. The insect is now generally distributed throughout the State of Pennsylvania to the west and through most of the State of Maryland to the south. Additionally, isolated infestations now exist in the States of Ohio, North and South Carolina, Virginia, West Virginia, Wisconsin, Michigan, and Washington.

Within the generally infested area, gypsy moth numbers fluctuate widely in time and place in almost unpredictable fashion and in response to a diversity of factors and processes. The insect possesses many attributes that appear to have furthered its survival and spread: High reproductive potential, ability to successfully feed on over a hundred species of trees and shrubs, and a variety of morphological and behavioral traits that enhance survival.

When at defoliating levels, the gypsy moth causes significant ecological effects and economic impacts in both forest and urban environments. The gypsy moth has always been a people problem in North America. The first outbreaks encompassed forested lands that included 30 towns and cities in the greater Boston area; the extensive defoliation and nuisance created by enormous numbers of larvae are vividly described in the early literature (Forbush and Fernald 1896). Since then, man has been responsible for the inadvertent transport of gypsy moth life stages that have resulted in the remote infestations that reoccur far beyond the generally infested area.

Human influence during the past 300 years has also undoubtedly changed the character of eastern forests and made them more susceptible to a number of pests, including the gypsy moth. Practices such as land clearing, heavy cutting, grazing, and indiscriminant burning have resulted in a network of forest stands that reflect different successional stages of disturbance (Smith 1976). More recently, urban sprawl, the encroachment of people and developments into once forested lands, has created situations that have apparently benefitted the insect. It is there at the forest/urban interface that the gypsy moth problem has been and will continue to be most severe.

Although the nuisance effect of the gypsy moth is still paramount in the minds of the general public, there is probably more public concern now than at the turn of the century about the effects of the gypsy moth on forest resources. As the gypsy moth gradually spreads to the South and West, it poses a threat to the commercial hardwood forests, although one can only speculate as to what effects the insect will produce there in the future. Likewise there is a growing public awareness about multiple-use forestry and the values associated with forested lands. A detailed discussion of the socioeconomic impacts caused by the gypsy moth and methods for their evaluation is included in chapter 7.

Historical Chronology

Many significant technological developments and precedents have been associated with mass attempts to eliminate, control, or contain the gypsy moth. The costs associated with these attempts are staggering, especially when the total expense is related to the value of the dollar at these points in time. Because the battle between man and the gypsy moth has been continual since 1890, a review of this important segment of entomological history seems appropriate for this compendium.

1869–1900

The original infestation increased and spread gradually until, by the summer of 1889, the insect was so abundant and destructive that it attracted public attention (Burgess and Baker 1938). The conditions were so serious that in 1890 the Massachusetts Legislature appropriated $25,000 for field operations to control the pest. The next year, the State embarked on a program designed to exterminate the insect from Massachusetts. Control operations consisted of: Applying creosote or acid to egg masses, burning infested trees and shrubbery, banding trees with burlap and sticky material to either trap the larvae or prevent their climbing the trees, and spraying with chemical insecticide (Kirkland 1905, Burgess 1930). Paris green, the chemical used initially, was replaced in 1893 with lead arsenate, a compound developed specifically for use against the gypsy moth. The availability of this arsenical precipitated the rapid development of and improvement in spray equipment and spray technology.

During this period most of the research was descriptive and centered on studies of the life history and behavior of the gypsy moth in the infested area. Intensive field studies that were devoted to observing the occurrence and behavior of vertebrate and invertebrate predators and parasites provided valuable baseline data for modern day researchers. Experiments were also conducted to mass-trap male moths with sticky traps baited with female moths so that unmated native females would deposit unfertilized egg masses. These attempts were not successful.

The control work from May 1891 to February 1900 was so successful in reducing the infestation that the Legislature chose to abandon the project. This action was considered by many to be a fatal mistake. Massachusetts spent an estimated $1.2 million during this period in its attempts to eradicate the gypsy moth.

1901–1919

During the next 5 years, gypsy moth populations increased tremendously in Massachusetts, and new infestations were discovered in Rhode Island (1901),

New Hampshire (1905), Connecticut (1906), and Vermont (1912). Massachusetts resumed control activities in 1905, and in 1906, Congress first provided funds to aid the State with its control and containment efforts.

From 1906 to 1912, the Federal Government and Massachusetts jointly financed the importation of natural enemies of the gypsy moth from several European countries and Japan (Brown and Sheals 1944). Intensive studies were conducted to learn more about larval dispersal but especially to determine the maximum distance that the newly hatched larvae might be windborne (Burgess 1913, Collins 1915). Some of the earliest research on the gypsy moth wilt (virus) disease (Glaser 1915) and on managing infested woodlots (Clement and Munroe 1917) was conducted during this period. Efforts designed to prevent the shipment of infested products into outlying areas were intensified. This, coupled with widespread concern that the insect would spread unimpeded unless delaying tactics were employed, resulted in the enactment of a Federal domestic quarantine against the insect in 1912. The quarantine remains in effect today and is credited with greatly reducing the accidental long-range transport of gypsy moth life stages on regulated commodities.

Between 1906 and 1920, the gypsy moth spread westward at an estimated rate of 9.6 km a year. Isolated infestations were discovered in 1913 at Geneva, New York, and in 1914 on estates at Cleveland, Ohio, and Westchester, New York. By 1914, the generally infested area included the southern half of New Hampshire, Rhode Island, eastern Connecticut, southern Vermont and Massachusetts east of the Connecticut River.

1920–40

In 1920, a serious infestation covering 1,040 km² was discovered near Somerville, N.J.; this apparently resulted from a separate introduction of infested blue spruce trees from the Netherlands. This infestation was finally eradicated by 1931 at a total cost estimated at $2.5 million (Felt 1942).

By 1922, the insect had spread through New England to the New York boundary. At a meeting in 1923 in Albany, N.Y., members of the U.S. Department of Agriculture and representatives from infested States and Canada decided to establish a barrier zone extending from Canada to Long Island, largely along the Hudson River and Champlain Valleys, encompassing some 27,300 km². The purpose of the barrier zone was to prevent the westward spread of the gypsy moth through a cooperative effort by the Federal Government and the State of New York. Infested territory to the east of the zone was to be treated by the States and supplemented by the liberation of parasites and other natural enemies by the Bureau of Entomology. All infestations found within and to the west of the barrier zone were to be eradicated.

In older infested areas, conditions had improved by 1920, and in the next 4 to 5 years, defoliation in New England was at the lowest level since 1905. However, heavy defoliation occurred on Cape Cod in 1925-26, and the reinfestation of old areas continued. The first aerial spray contract for gypsy moth control was awarded in 1926 on Cape Cod, Mass.

Research was intensified on natural enemies of the gypsy moth (especially parasites) and on the effects of defoliation on trees and forests; this resulted in a substantial increase in the quality and quantity of published material on the insect. A number of plots were established in Connecticut and Massachusetts to study the population dynamics of the gypsy moth under a wide range of woodland conditions (Bess 1961). This was one of the first major undertakings of its kind.

Spot infestations occurred annually within the barrier zone and were subsequently reduced or eliminated through intensive control efforts. However, in 1932 a serious infestation was found in the Wilkes Barre–Scranton, Pa., area, far beyond the barrier zone. The main infestations covered a 39 km² area, but smaller infested spots were later found over a 2,600 km² area in five counties.

A cooperative Federal-State eradication effort was begun in 1932, utilizing the same methods used earlier in New England. Although spot infestations were eliminated, the gypsy moth persisted in Pennsylvania. Meanwhile, the barrier zone became generally infested by 1939.

1941–60

By 1941, regular and emergency Federal appropriations for maintenance of the barrier zone were reduced substantially, resulting in the termination of the total effort. Felt (1942) prepared a position paper that strongly endorsed the renewal of efforts and funds to maintain the barrier zone; he projected that if the gypsy moth was allowed to spread unimpeded and to become established throughout the range of white oak, annual control costs for ornamental trees alone might easily approach $90 million. In addition, defoliation on forested lands might reach 10.1 million ha annually. It is estimated that the total effort through 1941 to eliminate the insect from Pennsylvania cost approximately $4.5 million.

The insecticide cryolite was applied experimentally in Pennsylvania in 1943 against the gypsy moth but gave unsatisfactory control. In 1944, the War Department allotted about 45 kg of DDT to determine its value in gypsy moth control and eradication work in Pennsylvania. Experimentation with DDT in Pennsylvania continued until 1948 and resulted in the development of modern methods of application such as airplane spraying and the mistblower. The Pennsylvania infestation was supposedly eradicated by 1948; however, two undetected infestations remained, and the State has been subject to new infestations and continual spread of the gypsy moth since that time (Nichols 1961)

Gypsy moth infestations seemed to explode in 1951–52, and in 1953, over 0.6 million ha were defoliated (25–100 percent) in the Northeast. A thorough appraisal of the gypsy moth problem was undertaken in 1952 for the purpose of developing a coordinated plan for the eradication and/or control of the insect in the United States (Perry 1955). The consensus was that the Adirondack Mountains in New York and their extension into the Allegheny

plateau presented the only natural ecological barrier to prevent the continued spread of the insect to the South and West. A seven-point plan was formulated to reestablish the barrier zone, operate a cooperative survey and eradication program within and to the east of the barrier zone, conduct a cooperative survey and control program with States east of the barrier zone, initiate trapping surveys with cooperators south and west of the barrier zone, provide Federal technical assistance to States to develop control techniques and programs, strengthen quarantine operations, and establish study plots in the generally infested areas to gain information on the epidemiology of the gypsy moth. The plan was approved in 1953 by the Regional Coordinating Committee on Gypsy Moth control of the Council of State Governments and put into operation within the limits of then available funds. The States were also encouraged to urge the Congress to appropriate funds necessary to carry out the proposed program.

Between 1953 and 1957, the insect was detected in an estimated 3.6 million ha previously uninfested in New York, New Jersey, and Pennsylvania. In addition, an infestation was found near Lansing, Mich., and was chemically treated in 1954. In 1956, the Congress made funds available to initiate an eradication program. During that year, 222,000 ha were treated with DDT in northern New Jersey, southeastern New York, and northeastern Pennsylvania. This was the first phase in a scheduled long-range program to eradicate the gypsy moth from the United States. If after 2 or 3 years this objective was found to be not feasible, the minimum goal would then be to eradicate all infestations back to the barrier on the Connecticut-New York line. Over 1.2 million ha were sprayed aerially with DDT in 1957.

About this time, the question of residues of persistent pesticides, such as DDT on food and feed crops, began receiving increased attention. There was also increasing concern about the effects of chlorinated hydrocarbons on certain species of beneficial organisms, fish, and wildlife. By 1958, a decision had been made to phase out DDT and replace it with carbaryl (Sevin®) as the chemical of choice. This was the last year in which DDT was used for control of gypsy moth.

With the exception of the classical work by Bess et al. (1947), the overall level of research was minimal during this period and is reflected by the relatively small number of publications to be found in the scientific literature.

In 1958, defoliation by gypsy moth was recorded on only 50 ha within the total infested area and most of that occurred in Connecticut. This was the first time since 1924 that fewer than 400 ha of defoliation were recorded for the region inhabited by the gypsy moth.

1961–70

By this time hopes to eradicate the gypsy moth were abandoned, and a long-overdue emphasis was placed on research. Research on alternatives to control of the gypsy moth had languished for two decades, during which time the pesticides DDT and Sevin® were extensively used. Studies intensified on two microbial pathogens, Bacillus thuringiensis (Bt) and the natural nucleopolyhedrosis virus (NPV). Laboratory and field trials were initiated to evaluate the sterile male technique and the new synthetic pheromone, gyplure. These initial studies provided valuable baseline information for the period of intensive research that followed in the 1970's.

Meanwhile, the amount of defoliation increased steadily beginning in 1959 in New England and New York. Following a brief respite in 1966–68, populations exploded in 1969 throughout the Northeast, and for the first time, heavy defoliation was recorded simultaneously in New Jersey and Pennsylvania. The developing outbreak focused attention on the need for a strong continuing research effort on the gypsy moth.

An ad hoc Federal-State committee, later to become the National Gypsy Moth Advisory Council, was formed; in 1969, its first official meeting was held in Washington, D.C., with a U.S. Senator from Pennsylvania in attendance. Several committees were formed; one, the Action Committee, immediately

began to consider strategies for obtaining additional research and control funds.

After another council meeting in Washington, D.C., in 1970 with prominent Federal-State delegates present, it was subsequently announced that additional funds would be allocated for research and control. Planning was initiated for a 5-year accelerated research and development program.

The Accelerated Program, 1971-74

In 1971, the U.S. Department of Agriculture redirected $1 million for research, much of which was initially designated for cooperative studies with universities and various State agencies. Additionally, the Forest Service and the Agricultural Research Service (now Science and Education Administration—Agricultural Research) increased base funding and resources for research and development. The following major areas of emphasis were identified— development and evaluation of the new synthetic sex attractant, disparlure; increased foreign exploration for parasites and predators; developmental research on microbial controls; and increased efforts to analyze and predict both changes in populations and their effects on the environment.

Meanwhile, the gypsy moth situation worsened. Over 400,000 ha annually were defoliated in 1971-73, with the highest level in history—800,000 ha—recorded in 1971. The States of Connecticut, New York, Pennsylvania and New Jersey incurred the most damage although the outbreak was truly regional in scope. In New Jersey, oak mortality increased dramatically after repeated defoliation in some areas and was reminiscent of the situation in Massachusetts in the early 1900's.

The Expanded Program, 1975-78

The severity and scope of the gypsy moth problem in the early 1970's, concurrent with situations with the Douglas-fir tussock moth in the Pacific Northwest and the southern pine beetle in the South, created both local and national concern. The Department responded by planning and initiating the U.S. Department of Agriculture Expanded Gypsy Moth Program as part of the Combined Forest Pest Research and Development Program (CFPP) (Ketcham and Shea 1977).

The expanded program was designed to complement the accelerated effort already underway and to achieve specific objectives and accomplishments within a definite time frame—4 years. In the planning process, objectives were clearly stated and well defined. Logically conceived plans to reach objectives were outlined, but adequate flexibility was provided to account for the uncertainties of research. All research activities were organized on a chronological schedule that provided for an orderly system of annual progress. The goals for the expanded program as set forth in Congressional Hearings and the resultant appropriation bill were as follows:

1. Methods for predicting population trends will be updated and refined using new techniques for measuring larval dispersal, sampling egg masses and pupae, and monitoring low populations.
2. Procedures for measuring and predicting impacts will be developed by refining methods for measuring defoliation, relating defoliation to tree mortality, and developing additional technology for measuring socioeconomic and environmental impacts.
3. Safety and efficacy tests to evaluate and support registration of nucleopolyhedrosis virus (NPV) will be completed.
4. Optimum formulations and application technology for use of chemicals, *Bt*, and NPV will be developed.
5. Disparlure's use in containment, suppression, and possible elimination of populations will be demonstrated.
6. New chemical insecticide candidates will be screened, evaluated in the laboratory, and field tested.
7. The effectiveness of available and newly introduced parasites will be evaluated.

8. Sterile male techniques will be evaluated for their potential as suppressive or population elimination tools.
9. A mass-rearing capability adequate for future program support will be developed.

The ultimate goal of the program was to incorporate this emerging technology into an integrated pest management system to address the total gypsy moth problem both behind and beyond the advancing front of the infestation.

Technology Transfer: the Compendium

As mentioned in the Foreword, much consideration and discussion was devoted to the subject of technology transfer or presenting program products to the ultimate users. One of the vehicles selected to accomplish technology transfer was this compendium.

A meeting was held in May 1977 attended by representatives of the four Federal agencies involved in the gypsy moth program—Forest Service, Animal and Plant Health Inspection Service, Agricultural Research Service, and Cooperative State Research Service—the program management team, and scientists designated as chapter coordinators for the compendium. A format and guidelines for the compendium were established: The tone should be semitechnical; methods and procedures should be kept to a minimum unless unique to the literature; contributors were encouraged to include a literature review sufficient to set the stage for their own research and to provide readers with a convenient reference to the pertinent literature on that subject; and principal investigators should have the opportunity to submit manuscripts covering their research findings and interpretation of results subject to technical and editorial reviews.

It was desired of both program management and the investigators involved that this compendium should be a true state-of-the-art document on the gypsy moth in the United States at this point in history emphasizing the research results obtained during and prior to the expanded program. Many of the funded investigations conducted during the period 1975–78 were continuations of research initiated during the period of acceleration in 1971–74.

This compendium is not all inclusive; for example, it does not include research funded by the Expanded Gypsy Moth Program that was and is being conducted by various States, universities, agricultural experiment stations, and private industry. However, in most cases these accomplishments are referred to in the references cited section for each chapter.

We hope that this volume is interesting and understandable and trust that it will serve as a valuable reference to the rapidly accumulating body of knowledge about the gypsy moth and its related effects upon the environment.

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Bioecology of the Gypsy Moth

David E. Leonard

Foreword

The vast majority of the millions of species of animals that share our planet are innocuous. Some of the most notable exceptions, however, can be found among the insects, the most successful of all organisms in terms of number of species, diversity, and adaptiveness. Other than the oceans, there are few places that man can exploit that are not being exploited by insects. Of the 5 million or so insect species, about 10,000 are considered pests because they directly compete with man for food, fiber, and living space, or act as carriers of diseases to man or domesticated animals. Relatively few of the pest species, however, are of such importance that they become household names, as has the gypsy moth. Lest we feel that our preoccupation with the gypsy moth in North America is unique, it should be noted that common names for this insect exist in virtually every language in the temperate regions of the world from North Africa to Japan. It is ironic that the name gypsy moth comes from the British Isles, where the insect is now apparently extinct.

The information that has accumulated on the gypsy moth is vast and varied, ranging from accounts of public hysteria to reports of basic scientific research that form the cornerstones of concepts and principles of such important biological disciplines as genetics and chemical communication. The purpose here is to provide an overview of the biology and ecology of the gypsy moth, with emphasis on findings obtained during the Expanded Gypsy Moth Program. This synthesis has been gleaned from many authors; more detailed accounts of most of what is presented here can be found in the other chapters of this book, along with credits due those people who provided the information.

The gypsy moth and many humans share a preference for the same habitat. Although the gypsy moth existed many millenia before man, most humans consider the insect an intruder and view it with decidedly negative thoughts. One can sympathize with the tribulations of those living in the midst of an outbreak of the gypsy moth; at the same time, however, one must appreciate this insect for what it can do and how it does it. Evolution has served the gypsy moth well; it is no easy foe.

Introduction

The gypsy moth, *Lymantria dispar* (L.), is in the order Lepidoptera, which contains the moths and butterflies, insects known for their beauty and, in some instances, for their destructiveness. Lepidopterans are characterized by having a complete metamorphosis, with larvae transforming to pupae, then metamorphosing into adult butterflies or moths. The gypsy moth is in the family Lymantriidae. Lymantriid larvae, which feed on tree foliage, are commonly called tussock moths because of the prominent tufts of hairs on the larvae. Representatives of this family are found in temperate regions throughout much of the world, but the gypsy moth is not indigenous to North and South America. This insect is found from northern portions of Africa through western and eastern Europe. Although the range of distribution is commonly extended through the Soviet Union and Japan, morphological, behavioral, and genetic differences, particularly in the forms from Japan, indicate that the Asiatic and probably the Eurasian gypsy moths are different species.

In its native range, the closest relative of the gypsy moth is the nun moth, *Lymantria monacha* (L.). The nun moth is common to higher elevations in Europe, where it feeds on needles of conifers. The gypsy moth and the nun moth differ in appearance and in their choice of food plants, and their ranges of distribution overlap only where conifers and hardwood host trees coexist. Interestingly, females of the gypsy moth and nun moth utilize the same chemical, a sex pheromone, to attract males for mating, attesting to their close relationship, but females of each species differ in the time of day that they release pheromone.

The gypsy moth has the distinction of being the first of three species of lymantriids to be introduced into North America in 1869 from Europe. The other two species are the browntail moth, *Euproctis chrysorrhoea* (L.), introduced about 1890, and the satin

9

moth, *Stilpnotia salicis* (L.), introduced about 1910. Although the gypsy moth has emerged as the most successful colonizer, the initial success and spread of the browntail moth were more spectacular. This insect defoliated many of the same species of trees as the gypsy moth, spreading throughout New England and into several of the Canadian Maritime Provinces in about 30 years. In addition to its widespread defoliation, the insect posed a very real public health hazard, for the hairs or setae contain urticating (irritating) properties that produce a serious rash and in several instances have caused death. For reasons not completely understood, the range of the browntail moth began to recede in the 1920's. Today, the insect is a maritime curiosity, with small colonies that persist on several islands in Casco Bay, off Portland, Me., and on coastal sand dunes of Cape Cod, Mass.

The satin moth was introduced into Northeastern and Northwestern North America; its larvae prefer a narrower range of host plants, species of poplars and willows. It is now considered a minor pest of ornamental poplars and willows, although there are infrequent outbreaks in forests.

The introduction of an insect to a new area by man, often with serious consequences, is unfortunately not uncommon. In some ways, however, the introduction of the gypsy moth into Massachusetts was different, for the insect was purposely imported in an attempt to develop a silkworm industry in North America. Perhaps Professor L. Trouvelot, an astronomer and naturalist, can be faulted for dabbling in biology in his attempt to interbreed gypsy moths with silk worms, but the tenants of biology were less well known in 1869. One could not fault him for the concern he showed in his prophetic oral and written warnings of the possible consequences of the accidental release, yet he could muster no officials willing to provide assistance to search and destroy the liberated gypsy moths. From what might be considered a comedy of errors originating at 27 Myrtle Street, Trouvelot's residence in Medford, one disquieting fact remains: Some gypsy moths escaped, and although the numbers were undoubtedly small, the social and economic costs engendered by the few survivors have been enormous.

The record of the establishment of the gypsy moth and the early spread of the insect is well documented in the book, *The Gypsy Moth,* written by Forbush and Fernald and published in 1896. For about 10 years after its release, gypsy moths were noticed only as a nuisance by those living near Trouvelot's old residence. The first apparent outbreak occurred in 1889 in Medford, and since then, the insect has not escaped public notice. In 1890, an act was passed in the Massachusetts Legislature appropriating $25,000 and forming a commission of three persons to oversee the extermination of the gypsy moth, the first of many such actions reenacted in a series of legislatures with ever-increasing dollar amounts. The 1890 act and those that followed were initiated too late after the establishment of the gypsy moth to eradicate it or to prevent its spread.

Small larvae, upon hatching from eggs in the spring, are well adapted to be carried on currents of air. The unfed insects, light in weight and clothed with long hairs that increase their surface area, hang on silken threads that they have spun. When the wind blows, the silken threads break and the larvae are carried aloft, helped by the long hairs and the silken thread, which increase the insects' ballooning capabilities. The early spread of the gypsy moth was primarily to the North and East, by the prevailing winds which blow from the Southwest.

The gypsy moth continues to spread into new areas, primarily south and west. Although the natural spread is rather slow, the inadvertent transportation of various life stages, particularly eggs, on transportable items as recreational vehicles, has resulted in establishment of isolated gypsy moth populations in regions well beyond the Northeast.

Life Stages

Adults

The species name of the gypsy moth, *dispar,* is derived from the Latin word that means *to separate* and accurately depicts the contrasting appearance of male and female moths. The males are mottled brown in color (fig. 2–1), with black wing markings; female

moths are white or cream colored with distinctive black markings on the wings (fig. 2–2). Other differences are found in the antennae, with males having plumose or feathery antennae, in contrast to the thin female antennae. The body of the female is much more robust, the abdomen a sack full of eggs.

Moths emerge from pupae in midsummer, usually in July, but the date of emergence can vary among areas in response to climatological differences or effects of density. Where population densities of larvae are high, development is often accelerated. Moths are able to commence activity shortly after emergence from the pupae; the soft-bodied adults orient with their heads upward, pump air into veins to expand their wings, and remain quiet for about an hour until the wings have expanded and the soft cuticle has hardened.

Males usually emerge 1 or 2 days before females. The flight of male moths has been characterized as a flutter, or zigzag, but males are also capable of rapid direct flight. Most flight activity occurs during hours of light, with males strongly attracted to vertical objects. They commonly fly up and down tree trunks where females would most likely be encountered. The dramatic response of males to the female sex pheromone is well documented. The feathery antennae of the male provides a large surface area for receptor cells, and it has been hypothesized that a single molecule of the pheromone impinging on a single receptor cell on the antennae is sufficient to elicit a behavioral response.

An important behavioral difference between sexes occurs with the flight capabilities of females. Although their wings are fully formed, females do not

Figure 2–1.— *Male gypsy moth. Note mottled brown color.*

Figure 2–2.—*Female gypsy moth. Note distinctive black wing markings.*

fly, a rather frequent adaptation in insects to shunt energy reserves normally expended in flight into increasing the reproductive capacity. In parts of Eurasia and Asia female flight is common, but these forms may be different species than *L. dispar.*

Several hours after emerging, females begin releasing pheromone from small glands near the tip of the abdomen. The release of pheromone is associated with the exposure of the terminal segments of the abdomen and a rhythmic pumping motion, an activity that has been termed "calling." The pheromone is released in bursts of calling activity, rather than continuously throughout the day. The biological perfume can attract males for long distances, with the male following the odor trail in the air with a rather erratic flight pattern and tracking the increasingly strong gradient of the pheromone to its source. Once within several feet of the female, visual cues are also utilized to locate the female, probably aided by the contrast of the light-colored female on the darker substrate of the tree.

Courtship behavior is not elaborate. Once the male is alongside the female, mating will not occur unless the female lifts her wing to allow the male to couple. The pair remains in copula for up to an hour, but the passage of the spermatophore, or sperm packet, is usually accomplished in the first 10 minutes. Once mating is completed, females begin the task of depositing eggs.

Male moths can successfully inseminate several or more females. Multiple mating in females is less common, probably because a feedback mechanism stops the release of sex pheromone in response to mating.

The moths live for about a week. Although moisture is imbibed, the digestive system is not functional and no feeding occurs. The timespan for reproduction in females is shorter than a week. By about the third day after emergence, the attractiveness of the females is greatly diminished, perhaps because the supply of pheromone is exhausted. If a male has not been solicited by then, there is little likelihood that the female will mate. Since males emerge sooner than females, the probability is increased that receptive males will be available when females emerge, and the efficiency of the sex pheromone insures that most females will mate.

Eggs

A period of 8 or 9 months is spent in the egg. The female gypsy moth has one generation per year and usually deposits eggs in a single cluster. Although the process can take several days, most eggs are laid within 24 hours after mating. If interrupted during oviposition, a female may start a new cluster. Females that are not fertilized will oviposit, but the eggs are usually scattered singly or in small, disorganized clusters and do not hatch.

The eggs are covered by a dense coating of hairs, sloughed from the abdomen of the female as she oviposits. The appearance of the egg clusters has prompted several of the common names for the insect, including the German "Schwammspinner" (fungus spinner), because the egg cluster resembles a small tree fungus; and the French "la spongieuse," from the spongy texture of the egg cluster. The dense covering of hair provides protection from egg predators and parasites, and may be important in insulating eggs from cold temperatures and acting as a moisture barrier.

The number of eggs per female varies from fewer than 100 to over 1,000; where conditions are optimum, egg clusters average about 750 eggs, compared with about 300 eggs at the end of an outbreak when the population starts its precipitous decline.

Because females are flightless, egg clusters are commonly found within several feet of the empty female pupal cases. Generally, most egg clusters are found on the trunks of trees, often in places that are dark and provide shelter, such as in crevices, under loose bark, or beneath scaffold limbs (fig. 2–3). Not all individuals pupate on trees, however; egg clusters are frequently found on or under objects such as rocks, tree stumps, foliage, and vehicles.

Before pesticides were available, considerable efforts were made to eliminate the gypsy moth by

egg reduces its water content as a protection from freezing. In the spring, most likely in response to accumulated heat units as the temperature begins to moderate, activity is initiated and water is resorbed. The larva chews through the chorion of the egg and the dense mat of hair and emerges about the time the trees are beginning to produce new leaves.

Larvae

Hatch and activity of new larvae are strongly influenced by temperature. Most larvae hatch within a period of a week, but hatch can extend for as long as a month especially when egg clusters are deposited in cooler, shaded areas or at higher elevations such as mountainous regions.

Newly hatched larvae remain on or near the egg cluster (fig. 2–4) if they emerge during rainy weather or if temperatures are below 7° C. When they leave the vicinity of the eggs, larvae are positively phototrophic and negatively geotropic. As they move, they spin a thread of silk. Larvae from eggs deposited on trees will move up the trees to the branch tips. Larvae

Figure 2–3.—*Gypsy moth egg clusters, usually found in sheltered places.*

destroying egg clusters, at a considerable expenditure of man-hours. The density of egg clusters per unit area is the most reliable means for estimating population density and predicting population trends. Interestingly, dogs can be trained to the odor of egg clusters and have detected concealed clusters at the base of trees and in or under objects on the ground.

Egg embryonation begins soon after oviposition, and larvae are fully formed inside the egg in about a month. Development ceases in preparation for diapause. A small percentage of eggs hatches in the fall, but the larvae do not develop, although a nondiapausing strain has been selected in the laboratory from such individuals. The diapausing larva inside the

Figure 2–4.—*Newly hatched larvae.*

that emerge on inanimate objects will climb upward; on reaching the top of the object they must rely on the wind to remove them, for they do not have the propensity to climb downward to depart unsuited places. Many larvae apparently disperse, even if suitable foliage is available. Dispersal is a critical episode in the biology and ecology of the gypsy moth and is discussed in more detail in chapter 4.

Larvae begin feeding on new leaves, first on the leaf hairs, then on the leaf epidermis, where small holes are cut then on the leaf margins (fig. 2–5). Feeding occurs during the daylight hours, particularly early in the morning after temperatures moderate, with another peak of feeding late in the afternoon. This feeding rhythm will change as larvae grow older and feeding becomes a nocturnal activity. When not feeding, the small larvae remain on the underside of the leaf along the midrib, where they form a mat of silk produced by

the silk glands and spun from spinnerets located near the mouth. The silk is important in providing larvae with a point of attachment to the leaf and preventing dislodgement.

The small size of the larvae at hatch, about 3 mm, belies the ultimate size of the larvae when development is complete, about 50 to 90 mm, with over a thousandfold increase in weight. To grow, larvae must molt. In preparation for the molts, which occur at about weekly intervals, the larva ceases feeding, deposits more silk to reinforce the silken mat, and voids the gut. The old cuticle splits, and the larva crawls out. The new cuticle requires several hours to harden after the molt. The membranous portion between the larval segments is elastic and will stretch as the insect feeds, permitting expansion and growth.

Larvae are normally characterized by instar, determined by the number of molts. A newly hatched

Figure 2–5.— *Larvae feeding on new leaves.*

larva is in the first instar until the first molt. A third-instar larva has molted twice, a fifth-instar larva four times, etc. The gypsy moth larva displays considerable plasticity in the number of instars it undergoes before reaching the pupal stage. Generally, males have five instars (four molts) and females, six instars, but one additional instar is common for both sexes, and as many as nine instars have been recorded in laboratory rearings. Molting is controlled by the hormonal system, which is affected by a number of variables, including nutrition.

Growth and development of larvae are influenced by physical factors such as temperature and moisture, and by biotic factors, such as quantity and quality of food and vigor or quality of the individual insects, as discussed later in this chapter.

Larvae usually remain on the leaves during the first several instars. Fourth- and some third-instar larvae seek resting sites other than the leaves when not feeding; it is at this point that they display a dramatic shift in their diel rhythm, with feeding switching from a day to a night activity. Using the decreasing light levels at dusk as their cue, larvae vacate their resting sites and move up the tree, following trails of silk deposited during their daily sojourns. Feeding can occur throughout the night, but a prolonged peak of feeding occurs after the larvae reach the foliage in the evening, with a smaller peak prior to dawn. With the light of the new day, larvae retrace their paths to the resting sites.

The feeding of small larvae is hardly noticed, for they do not consume much foliage. As larvae grow, however, they consume increasing amounts, and in the last instar they eat more than in the other stages combined (fig. 2–6). Last-instar female larvae are the most voracious feeders (fig. 2–7); they are considerably larger than males and weigh over twice as much, and their last instar is longer in duration than that of males. It is estimated that a larva consumes about 1 m^2 of foliage during development.

The selection of resting sites can have a significant impact on survival, particularly in sparse populations. These sites are usually on the tree where feeding occurs—darkened areas such as crevices in the trunk, under loose bark, or on the underside of scaffold

Figure 2–6.—*Last-instar male larvae.*

limbs near the trunk. If no suitable sites are located on the tree, larvae will seek shelter nearby in the leaf litter, in rocky areas, or even on adjacent snags. Larvae usually utilize the same resting sites during their development, and this is where molting and pupation occur.

As the end of the larval period approaches, feeding is terminated. Larvae void the gut, surround themselves in a sparse silken net, and begin to contract in length. This prepupal stage lasts only for about 2 days, with prepupae remaining relatively quiescent inside the silken net.

Pupae

The prepupal stage is terminated when the cuticle splits along the midline of the dorsum and the pupa works its way out of the larval skin. The pupa turns from a whitish color with a greenish cast to dark brown within the hour or so that it takes for the cuticle to harden. The tear-shaped pupa resembles neither the larva nor the moth. Occasionally, gyrations of the pupa can be observed, particularly if the pupa is

Figure 2–7.—*Last-instar female larva; note larger size.*

disturbed. The pupa remains cradled in its sparse silken cocoon (fig. 2–8) for the 2 weeks (16 or 17 days for females) required for morphogenesis. When development is complete, the adult takes in air, expands, and splits the pupal skin. The moths crawl out fully formed except for the wings, which are quickly filled with air through the trachaea and puffed out like small balloons. After the several hours required for wing expansion and hardening of the cuticle, the moths are ready to carry out their function of providing a new generation of gypsy moths. The development of gametes in both sexes began in the later larval stages. Thus, in newly emerged moths, the sperm is viable and the eggs are ripe and need only to be fertilized as they pass through the oviduct.

Ecology

Hosts

The number of gypsy moth hosts exceeds 300 species of trees and shrubs, with species of oaks ranked among the most favored foods. Small larvae are more restricted in their host range, but as development progresses, the acceptable host range expands. Oaks are common components in many of the forests of North America, and their wide distribution will be a major factor in the ultimate range of the gypsy moth in this hemisphere. Where oaks are less common, however, as in boreal forests, the gypsy moth has established and

maintained populations on other tree species, including trembling and bigtooth aspen.

Some less favored host species, such as red maple, are fed upon when larval population densities are high and favored foliage is scarce. Under such conditions, larvae will consume nearly any foliage available, with only a few species, such as tulip poplar and dogwood, immune to feeding. Other species, particularly conifers, are not acceptable to early-instar larvae, but late-instar larvae feed readily on the needles of hemlock, balsam fir, and many species of spruce and pine.

The effects of the quality of the host foliage on gypsy moth survival and fecundity are just beginning to be appreciated. Trees under stress such as drought contain higher levels of nitrogen, which may provide larvae with an enriched food source. These findings provide important leads to understanding why the largest outbreaks of the gypsy moth are correlated with periods of below average summer rainfall, and the observations that the foci of epidemic populations are often on dry, rocky ridges where conditions for tree growth are not optimum.

Damage

The gypsy moth is a relatively large insect, with a big appetite. The number of larvae per tree is high when outbreak populations occur, and most or all foliage can be consumed (fig. 2-9). The insect is

Figure 2-8.—*Gyspy moth pupae.*

Figure 2-9.—*Defoliated trees.*

episodic, with extensive defoliation occurring during the last years of the outbreak phase. In Europe and currently in much of New England the gypsy moth is notorious as a nuisance and not for killing large numbers of trees, but this is not the situation when the gypsy moth first moves into an area. Historical records from New England and current data from New Jersey and Pennsylvania show that tree mortality is often extensive. Species of oaks (especially white and chestnut oaks, which appear to be the most susceptible) incur the highest percentage of mortality, often exceeding 50 percent of the dominant and codominant trees, with higher percentages of mortality in the understory trees. Because the hardwoods in the Northeast are not being heavily utilized for forest products, tree mortality has been less of a concern than the loss of the use of recreational areas. This is not the situation, however, in such areas as the Appalachians, where concerns for the hardwood forests are being voiced in anticipation of the imminent invasion of the gypsy moth.

To understand why the gypsy moth has had a greater impact in North America than in Europe, it is important to recognize that the insect is an invading species. When a species reaches a new habitat, the following can occur: (1) It becomes extinct, which happens in most instances; (2) it establishes and, although it reaches high numbers initially, ultimately reaches low population levels, often in very restricted habitats, as happened with the browntail and satin moths, both closely related to the gypsy moth; or (3) it establishes and goes through a rapid expansion of its range, as has occurred with the gypsy moth and other species such as the Japanese beetle, *Popillia japonica*.

What happened when the gypsy moth reached this continent? It found the climate and the nearly contiguous forests, some predominantly oak, to be favorable. Many of the trees are close relatives of species in Europe where the gypsy moth and hosts have coexisted for millenia, leading to the coevolution of adaptations that prevent or retard the potential elimination of the host or the insect. In North America, the tree species have evolved no such protective adaptations. Furthermore, the gypsy moth arrived here without its complement of natural control agents—parasites, predators, and disease. Although some native animals such as birds and several species of parasites fed on the insect, their effectiveness was much less than the population pressures exerted by natural control agents in the native range of gypsy moth.

Competition from other organisms utilizing the same resource is often an important factor in regulating population density. The gypsy moth, an early-spring defoliator, found no ecological homologs in North America that were its equal. Most competition came from other gypsy moth larvae, and under such conditions, populations usually increase until a critical factor becomes limiting, usually the amount of available food.

The net effect of all the above was what could be expected when factors regulating population numbers are relaxed or missing. Gypsy moth populations reached very high numbers and remained there for longer than the usual 2 years; the normal cyclic rhythm was lost. The effects of repeated defoliation can be devastating. Coniferous trees die after a single defoliation. Deciduous trees can withstand one or two defoliations, but the incidence of mortality rises sharply after the third defoliation. After the first defoliation, deciduous trees flush a new set of leaves in July, several weeks after larval feeding has completed. The new leaves, smaller and photosynthetically less efficient than the defoliated ones, are produced at considerable cost to the tree in terms of utilization of stored food resources. Although the trees put on little if any growth during the season, they usually survive until the following spring. The second defoliation stresses the tree even more, and only the hardiest survive a third defoliation. Other stress factors such as drought or poor site conditions decrease the chances for survival after defoliation. Understory trees usually suffer the highest mortality, but few of these would normally survive to become dominant or codominant trees. Most of the tree mortality is caused by pathogens or insects such as the twolined chestnut borer that attack and kill weakened trees.

Defoliation, however, can also benefit trees, as scientists are beginning to discover. Trees produce foliage in excess, and the removal of a portion of that

foliage can stimulate increased production in the tree. Furthermore, much of the available nutrients are bound up in the woody portions of the trees, slowing the recycling of nutrients. The foliage passing through the insect gut is broken down into a form that rapidly releases nutrients to the soil.

Nuisance Factors

Thousands of species of insects feed on trees, but few attain the gypsy moth's notoriety. There is a Jekyll-and-Hyde personality in the gypsy moth, with population density the potion that changes larvae from innocuous to obnoxious. At low densities, large larvae remain inactive and secluded in their resting sites during the day, but at high densities, the tranquil state changes dramatically. For reasons not yet understood, larvae in dense populations become hyperactive during the day. Wooded areas teem with larvae incessantly moving up and down trees. When larvae reach open areas, they "bee line," using polarized light to direct them in their straight path. These larvae are strongly attracted to and climb any object in their path—telephone poles, vehicles, fences, houses, and people. The larvae are rarely harmful, but few individuals find the presence of the swarms of larvae tolerable, particularly those with entomophobia. The larvae fortunately do not travel great distances.

There are several other factors that make gypsy moth outbreaks a public nuisance. As trees are defoliated, the normally cooler wooded areas warm; this loss of a cool habitat and the heat drive many animals from the woods, and some, like snakes, can become an annoyance.

When outbreaks occur, many larvae die from a variety of mortality factors and from one in particular, a nucleopolyhedrosis virus disease known as wilt. The unpleasant odor of decaying larvae often permeates the defoliated area.

The experience is distasteful for those who cannot avoid coexisting with gypsy moth outbreaks. There is little solace in the fact that populations usually reach high levels for only 2 years before the population collapses, although it can be longer in areas where the gypsy moth is extending its range.

Natural Enemies

Parasites

All organisms nourish a complex of parasites that serve as one means of regulating populations. It was fortuitous for the gypsy moth that it was introduced into Massachusetts without its parasites, but after success in controlling other insects with imported natural enemies, a similar program was started for the gypsy moth. Initiated in 1905 by the U.S. Department of Agriculture, in cooperation with several affected States, this effort has been the most extensive of all of the parasite introduction programs (in spite of several program interruptions) and has resulted in the successful establishment of 10 species of parasites and one predacious beetle. The sum total of mortality from these imported insects reaches high levels in many gypsy moth populations.

After a recent period of renewed interest, the search for parasites in Europe is being reduced, because it is thought that the most effective parasites from the area have been imported and released. The program has been expanded in Asia, however, and a parasite laboratory was recently established in Japan to ship potential parasites, predators, and pathogens to the United States for evaluation.

Entomophagous parasites kill their hosts. They usually develop inside of the host but do not feed on vital organs until they have nearly completed their development. Although some species of imported parasites have one generation per year, several are multivoltine. Most of the multivoltine species must attack hosts other than gypsy moth, for gypsy moths in the stage of development that these parasites attack are available for only a short period of time during each year. The abundance of these multivoltine parasites is, therefore, dependent on the number of available hosts. In some instances, parasites introduced into North America attacked gypsy moths but failed to establish because suitable alternate hosts did not exist.

Most of the parasitic species of insects are found in two of the most advanced insect orders, Diptera and Hymenoptera, of which members of both have been

successfuly introduced into this country. Their strategies in locating gypsy moths are quite different.

Parasitic flies, in general, rely on their acute vision to locate hosts. The four introduced species of flies attack the larval stages. One species, *Parasetigena silvestris*, locates large larvae, swoops in, and very rapidly lays an egg in the intersegmental fold. If the egg hatches before the larva molts and sheds the old cuticle, the larva penetrates into the host and begins its development. Another species, *Compsilura concinnata*, a multivoltine species, pierces its host and deposits its offspring, in this case a larva rather than an egg. A third, *Blepharipa pratensis*, deposits large numbers of eggs on the leaves of trees. This species apparently is attracted to a chemical or chemicals on freshly chewed leaves. The attraction to such chemical substances, termed allemones, increases the chances that eggs will be deposited near feeding larvae. To hatch, the egg must be consumed; if so, it hatches in the gut of the larva and begins its development. These three parasitic flies leave the host when development of the maggot is complete, burrow into the ground, and form a puparium from which the adult fly will emerge. *P. silvestris* and *C. concinnata* emerge from larvae, prepupae, or pupae; *B. pratensis* emerges only from gypsy moth pupae.

Exorista larvarum is the remaining parasitic fly that was successfully introduced. Like *P. silvestris*, it lays its egg on large gypsy moth larvae and, like *C. concinnata*, requires alternate hosts. It is less common than the other species of parasitic flies and overwinters as a maggot inside of alternate hosts.

The hymenopteran parasites use different strategies to find their hosts. Their eyes are not as developed as those of the flies, but their antennae are replete with large numbers of highly sensitive sense receptors. Chemicals provide important cues, and some species such as *Apanteles melanoscelus* and *Brachymeria intermedia* respond to host chemicals (kairomones) to identify gypsy moths. Kairomones also elicit egg-laying responses in the parasites.

Two species of wasps, *Ooencyrtus kuvanae* and *Anastatus disparis*, attack gypsy moth eggs. Since eggs are available for about 9 months during the year,

the effective parasitization of this stage could be important in regulating gypsy moth numbers. *A. disparis*, however, attacks only unembryonated eggs, limiting their period of attack to several weeks; furthermore, the female parasites do not have wings so the natural spread of the parasite is slow. *O. kuvanae*, which attacks unembryonated and embryonated eggs, has several fall generations and a spring generation. The parasite has all of the prerequisites to be an effective parasite except a long ovipositor. The ovipositor of *O. kuvanae* is too short to penetrate through a full egg cluster, and only the surface layers of eggs are parasitized.

Two species of wasps parasitize small gypsy moth larvae. *Apanteles melanoscelus* has two generations a year, with the first generation attacking first- and second-instar larvae, and the second generation attacking third- and fourth-instar larvae. The effectiveness of this parasite is limited by high overwintering mortality and by a large number of native parasite species (hyperparasites) that attack it. *Phobocampe disparis* also parasitizes early-instar gypsy moth larvae, but it is relatively rare and considered of minor importance.

Gypsy moth pupae are parasitized by *Brachymeria intermedia* and, with much less frequency, by *Monodontomerus aureus*. *B. intermedia* was the last introduced parasite to colonize, but once established, it spread rapidly. It prefers open, sunny areas, and high rates of parasitism are usually found in more open or defoliated areas. *M. aureus* is a primary parasite of both the gypsy moth and, more commonly, the browntail moth and a hyperparasite of hymenopteran and dipteran parasites. The value of this multivoltine parasite is questionable because of its rarity as a gypsy moth parasite and its negative impact on other parasites.

Several species of native parasites have been recovered from gypsy moths but rarely in high numbers. The hymenopteran, *Itoplectes conquisitor*, attacks and kills gypsy moth pupae, but few individuals successfully complete their development in this host.

Pathogens

A number of pathogenic microorganisms—viruses, bacteria, fungi, and microsporidia—infect the gypsy moth. By far the most important is the nucleopolyhedrosis virus (NPV) *Borralinivirus reprimens,* which causes polyhedrosis, or wilt disease, a name which aptly describes the flaccid appearance of dead larvae. This pathogen was thought to have been introduced with early shipments of parasites.

The epizootics of wilt disease are often spectacular, and mortality is most prevalent during gypsy moth outbreaks. When numbers of larvae are high, the spread of the disease is rapid. The viral particles, in bundles called polyhedra, must be consumed to be infective. Larvae can become infected by ingesting polyhedra on the egg chorion or in the hairs covering the egg clusters as they chew their way through to emerge. These infected larvae die in the first instar, and their bodies disintegrate, spreading viral particles on the foliage. Larvae consuming this foliage also succumb, continuing the process of spreading the disease. Gypsy moth outbreaks normally culminate with high amounts of mortality caused by NPV, bringing about a drastic reduction in population numbers. This pathogen has been registered under the name Gypchek for use as a biological insecticide for gypsy moth control, and an extensive program is in operation to produce quantities of the virus.

A strain of the bacterium *Streptococcus faecalis* is also an important pathogen, but its role as a mortality factor is often overlooked because of the more spectacular virus disease. Larvae killed by *S. faecalis* have a shrivelled appearance.

Predators

Unlike parasites that consume only one host, predators usually feed on a large number of hosts. Predators of the gypsy moth include insects, spiders, birds, and small rodents.

Calosoma sycophanta, a large, colorful beetle, is the only predator that successfully colonized of those species released in the parasite introduction program.

Both the larvae and adults of this predator are voracious feeders, using their large mandibles to tear open and feed on the contents of larvae and pupae. *C. sycophanta* can reach high numbers, with evidence of their rather gruesome attacks readily observed. The distribution of this predator tends to be spotty, however, and it has not been recovered in more northern regions of the range of the gypsy moth.

Some species of insectivorous birds prey on the gypsy moth. Although bird predation of eggs is not high (egg clusters appear to be protected by the hair covering), early-stage larvae are consumed by many bird species. Large later stage larvae, which are covered with long hairs, are consumed by a few bird species, including black-billed cuckoos. Flocking birds, such as grackles and blackbirds, have been observed to move into and feed in areas containing high gypsy moth larval populations. The effects of bird predation on North American gypsy moth populations have not been thoroughly studied. In Japan, however, the importance of bird predation has been documented, and in Eurasia, bird nesting boxes have been placed in infested forests to encourage the buildup of bird populations to reduce the numbers of gypsy moth larvae.

Rodents, particularly the white-footed mouse, *Peromyscus leucopus,* are considered important regulating factors in sparse gypsy moth populations. White-footed mice and other rodents such as shrews consume many of the large larvae and pupae that seek resting and pupation sites near the ground and in the leaf litter. Rodent predation is considered a major factor in preventing outbreaks in some areas where gypsy moth populations have remained relatively stable at low population densities for a number of years.

Factors Regulating Populations

All organisms are subject to both physical and biological factors that act to regulate population numbers. The availability and suitability of food, site

conditions, incidence of natural control factors, inter- and intra-specific competition, weather effects, and others criteria are major determinants in whether population numbers will increase or decline. Some of these factors are discussed in greater detail in chapter 4.

Climate Effects

The gypsy moth is strongly affected by climate— temperature, moisture, light, and wind. Also important, however, is the microclimate that occurs in the restricted habitats where the insect actually lives. For example, the microclimate of a secluded area beneath the bark where large larvae may congregate may be quite different from the climate of the surrounding area.

Temperature

Temperature is an important factor in gypsy moth development and survival. For eggs, low temperatures can be fatal. Larvae diapausing inside of the egg prepare for winter by reducing the volume of free water to prevent the formation of ice crystals, which can rupture cells and cause death. With much of the water bound up in glycogen, the insect can withstand temperatures of –9° C. However, exposures to this, or lower temperatures for extended periods will kill eggs, and –23° C for even a short period of time is lethal. In much of its native range and in northern portions of North America, the gypsy moth is exposed to temperatures that exceed the lethal limits. Interestingly, in these areas, survival is facilitated by behavioral modifications. Females deposit egg clusters on the ground or on the lower part of tree trunks, where they are well protected by the insulating property of snow. Periods of freezing temperature after hatch could have an effect on populations by killing the small larvae and/or the young leaves.

Exposure of developing larvae and pupae to constantly high temperatures in the 32° C range in the laboratory greatly accelerates growth and development, but the females reared under these conditions produce infertile eggs. This infertility probably does not occur in nature: Daytime temperatures often exceed 32° C, (with temperatures dropping at night), resulting in accelerated growth and development, but widescale outbreaks have been correlated with successive years of hot, dry weather during June. Accelerated development during such periods may increase survival by limiting the time larvae are exposed to mortality factors. Laboratory studies have shown that individuals that develop the fastest are larger, and larger females contain more eggs.

Moisture

In terms of rainfall or high relative humidity, moisture is also important. Heavy rainfall at the time of hatch can wash off and drown larvae that have not yet established feeding sites on the foliage. Periods of low populations, measured on a geographic scale by acres defoliated, are correlated with high amounts of rainfall during early larval development.

The effects of relative humidity on larvae have not been well documented. Many insects seek sites that best satisfy their preference for relative humidity, with some preferring a low range, others high, and some species displaying no apparent preference. Gypsy moth larvae, when consuming foliage, acquire considerable amounts of water, which must be eliminated, most probably through respiration. One might expect that larvae would not seek a microclimate where relative humidity is high, which would deter respirational water loss. The resting sites that larvae seek, however, tend to be dark, sheltered areas where relative humidities might be higher. The secluded resting sites probably afford better protection from parasites that rely mostly on vision to find their hosts.

Solar radiation is important, both for temperature and light. Gypsy moths respond to light in a diel rhythm of activity. As mentioned previously, small larvae feed during the day, whereas older larvae shift their rhythm to feed at night. Emergence of adults is also apparently triggered by the daily cycle. Larvae are strongly photopositive after they hatch and climb upward. Most feeding occurs in the tops of trees, with larvae moving downward when foliage becomes scarce. Gypsy moth larvae prefer trees on the margins

of the forest areas where tree canopies are more open. It is not known whether the insect is responding to increased amounts of light and higher temperatures, or whether the foliage produced under such conditions contains a higher concentration of nitrogenous compounds, hence providing the larvae with an enriched food source.

Wind

Wind is a critical element in the dispersal of small larvae. These larvae respond to air movement by arching their bodies, which releases their attachment to the substrate; silk may also be extruded. Small larvae are active during the daytime, when wind velocities are highest; a wind of only several miles per hour will break the silken thread holding the suspended larvae. Although the estimates of the distances larvae are transported vary, the importance of the dispersal of first-instar larvae is well appreciated as a prime mechanism for spreading infestations of the gypsy moth. Although a larva can undergo several dispersal episodes, the percent of mortality of dispersing larvae is probably very high.

Natural Control Factors

The percent of mortality caused by parasites, predators, and pathogens can be very high, and the rapid decline or crash of outbreak populations is often associated with a high incidence of these biotic agents, particularly NPV. There is some controversy, however, about the role of natural control factors, particularly parasites, in regulating gypsy moth populations. Although high amounts of parasitism are frequently reported, the gypsy moth continues periodically to reach outbreak levels. This situation, however, also occurs in the native range of this insect. The gypsy moth has evolved as an episodic insect, and although the effects of mortality-causing agents may reduce the amplitude of the outbreak numbers and may prolong the period between outbreaks, parasites cannot be expected to prevent the gypsy moth from cycling. The outbreak cycles provide for dispersal, with most numbers of larvae dispersing when population densities are high. Like most species of Lepidoptera, the gypsy moth quickly shows deleterious genetic effects from inbreeding, and dispersal provides more opportunity for genetic recombination.

The gypsy moth has a high reproductive potential that can result in numbers increasing manyfold from one year to the next. Parasites do not show a similar numerical response. The number of eggs most parasites are capable of depositing is usually much lower than the gypsy moth. Furthermore, parasites that develop only in the gypsy moth have evolved adaptations for surviving when gypsy moth numbers are low. To meet the increased energetic requirements for searching for scarce hosts, energy must be used for activities such as flight, and less energy is allocated to the production of eggs.

Some of the gypsy moth parasites have more than one generation a year and require alternate hosts. The abundance of these species available to parasitize gypsy moth is a function of the availability of suitable alternate hosts for the previous generation of parasites.

Pathogens, particularly the virus-causing wilt disease, can cause high mortality. Because of the nature of the spread of wilt disease, epizootics usually occur when larval population densities are high. A high incidence of wilt disease is associated with dramatic population crashes of the gypsy moth. Parasite and predator populations, which normally build during the outbreak phase, are important in reducing the residual gypsy moth population in the year following the crash.

Mortality factors that function against low host populations have the potential to prolong the duration between outbreaks. The most efficient of these appear to be white-footed mice and shrews. In habitats favorable for these rodents, their predation of larvae and pupae is considered the primary cause of retarding gypsy moth outbreaks in some areas.

The interactions of natural control agents and the variables of host density, quality, and climatic factors are complex. Because of the interactions of the large number of variables, future studies will require data to be collected in a form suitable for computer analysis and simulation modelling.

Effects of Density

Many insects respond to the density of their own numbers. In the gypsy moth, the effects of density or crowding are manifest in several important physiological and behavioral changes. Laboratory studies show that crowding of larvae accelerates development. This has been noted in field populations as well, with adults observed up to several weeks earlier in dense populations than in adjacent sparse populations.

Crowding of larvae during the first instar can induce additional instars. The crowding prolongs the period before larval feeding occurs, a critical period in development, since this is when dispersal occurs. The additional instars occur during the middle of larval development. Larvae with one or several additional instars take longer to develop into adults. This prolonged period of development would increase the exposure time of these larvae to mortality factors. Larvae with additional instars, however, eat more and produce larger adults, resulting in female moths that produce more eggs. This increase in reproductive capacity may help to compensate for a higher rate of larval mortality. The incidence of additional molting types provides an index of the quality or vigor of populations, as discussed in the following section on qualitative differences.

Color changes have been correlated with levels of crowding in some insects. This condition—phase polymorphism—is probably best known in migratory locusts. Gypsy moth larvae and adults respond to crowding with changes in coloration, with those reared under crowded conditions being lighter in color (fig. 2–10). Although this characteristic has been overlooked in North American gypsy moths, it may serve as a useful index for predicting population trends, as is being done in parts of the Soviet Union.

One of the more obvious effects associated with high larval densities is the wandering of large larvae during the day, a reversal of normal behavior. This swarming of larvae is, to many, more of a nuisance than defoliation, but it is an important larval survival factor in areas where food supplies are being depleted, because some larvae disperse to adjacent areas where food might be more plentiful.

Qualitative Differences

Since their effects are more obvious, climatic factors, parasites, predators, and pathogens are examined as elements that regulate gypsy moth numbers. It is becoming increasingly apparent that factors inherent in the insect itself must also be considered. Gypsy moth populations consist of individuals, and not all of these individuals are alike. Studies have shown considerable individual variation in survival, vigor, rate of development, behavior, dispersal potential, susceptibility to pathogens, and fecundity, underscoring the need to establish a profile of the quality of gypsy moth populations to provide more accurate predictions of population trends.

Qualitative differences are most frequently associated with the nutritional condition of the insect and provide the insect with the means to respond rapidly to environmental changes. The amount and quality of the food ingested and the utilization of the ingested food can be influenced by a number of environmental factors. The juvenile hormone system is affected by the nutritional condition of insects, and this system controls many important biological functions, including development, elements of behavior, fecundity, and the amount of food reserves (yolk) deposited in eggs.

In the gypsy moth, the incidence of larvae that have more than the expected four molts in males (five in females) is considered to be an indicator of a change in individual quality. An increase in the number of molts can be induced early in development by factors that also affect the amount of yolk in eggs, or the utilization of this food reserve by larvae. A survey of larvae reared from individual egg clusters showed that the number of larvae with one or more additional molts can vary from a few percent to nearly 100 percent. A strong maternal influence is shown in the amount of yolk deposited in the egg, with a positive correlation between small egg size (less yolk) and the incidence of additional molts. Thus, factors affecting larvae of the previous generation, when energy reserves for the yolk are accumulated, can influence the succeeding generation. The utilization of the yolk by the larva inside of the egg or after hatch prior to feeding can also affect the number of subsequent

Figure 2–10.—*Lighter color gypsy moths, a color change due to dense populations.*

molts. Factors that prolong the first instar, such as crowding, starvation, and cool temperatures, also cause additional molts. Although opinions differ on how the system functions, the quality of larvae emerging from the eggs as well as changes occurring prior to the first feeding affect larval behavior and dispersal. In other insects, where qualitative changes are known to occur, they affect dispersal. This provides for a rapid and efficient mechanism to respond to environmental factors such as crowding and results in self-regulation of population numbers.

In populations of insects that undergo a rapid increase in numbers when conditions are favorable, it has been speculated that a loss of genetic fitness occurs because of the accumulation of genes and genetic combinations that would normally be selected out of the population. Population crashes would result, in part, because of the deleterious effects of this genetic load. Such occurrences might happen with the gypsy moth, but at present, no genetic information exists on which to base such an analysis. Like most lepidopterans, the gypsy moth has a large number of chromosomes, and specific genes have not been located (mapped) on the chromosomes. The inheritance studies of Goldschmidt, although extensive, concern mostly the Japanese gypsy moth and its interspecific hybrids.

Influence of Man

One cannot discount man's influence on the gypsy moth problem, starting with the introduction of the

insect to this hemisphere, and the continuing accidental transport of the gypsy moth to new regions of the United States.

The alteration of the environment by man has also compounded the problem. Previous cutting practices have, in some areas, increased the percentages of oaks in forests. The thinning of forests for suburban homesites provides a more open canopy that favors gypsy moth development and survival. Signs on trees and posts, refuse, stone walls, etc., provide large larvae with protected sites that are readily utilized. A small increase in the percentage of survival of large gypsy moth larvae can have a marked influence on the size of the population of the next generation.

Pest Management

The ultimate goal of the Expanded Gypsy Moth Program is to develop sound pest management approaches to reduce gypsy moth numbers, to keep populations at low densities, and to retard the spread of the insect. The dictates of pest management are to keep population numbers at levels below some predetermined economic threshold, rather than to attempt to exert a high percentage of mortality. Because gypsy moth defoliation can occur in woodlands utilized for a variety of purposes— homesites, recreation, forest products, or unmanaged forests—the economic thresholds for tolerable defoliation will differ. Most of what follows in this book provides detailed accounts of research geared toward providing a pest management program; the following account is only a summary of some of the pest management tools now available.

Pesticides

Chemical pesticides provided the first effective means of reducing gypsy moth populations. Early treatments utilizing arsenical pesticides sprayed from the ground required much manpower and were limited to areas accessible to spraying equipment. The successes with aerial applications of DDT in the 1950's were most impressive, but the use of DDT for gypsy moth control was the cause célèbre in much of the early rhetoric concerning pesticides and the

environment. In retrospect, the use of DDT can be questioned, but it should be remembered that the early usage of this pesticide occurred before there was apparent reason for concern about the environmental effects and the accumulation of DDT and its magnification in the food chain were unknown. Ironically, one factor that made DDT so effective, its long residual effectiveness, caused its demise and the subsequent banning of similar compounds.

Pesticides still remain the most effective means of quickly reducing gypsy moth numbers, but the compounds now registered for use biodegrade rather rapidly and do not cause as severe environmental perturbations. Environmental monitoring accompanies all large-scale pesticide operations to reduce the possibilities of adverse environmental effects. Aerial application procedures have been developed to increase the coverage by sprays, which reduce the amount of pesticide applied per hectare.

Pesticides currently registered for gypsy moth control include carbaryl, a carbamate compound; Dylox, an organophosphate, Dimilin, and insect growth regulator; *Bacillus thuringiensis,* a bacterium that produces a toxic crystal; and the nucleopolyhedrosis virus (NPV).

Biological Control

Biological agents, parasites, predators, and pathogens all take their toll on the gypsy moth. A considerable amount of research, detailed later in this book, has been devoted to identifying the role of the biotic agents and, where possible, to enhancing their effectiveness. A search for new biological control agents continues in other countries.

There is an active cooperative Federal and State program to produce parasites in the laboratory and to introduce them in areas where the gypsy moth is spreading. In some States, parasites that have a wide host range have been introduced into areas where the spread of the gypsy moth is imminent, in an effort to establish them before the gypsy moth arrives.

The incidence of natural control agents is usually highest where gypsy moth population densities are high. Under these conditions, the biological agents,

with the possible exception of NPV, have the least effect on reducing host population densities. Although a high percentage of the hosts may be killed, surviving populations are often large enough to insure an increase in numbers in the next generation.

There are several ways in which the effectiveness of natural control agents might be enhanced. There have been some preliminary genetic studies to evaluate races of parasites from different geographic regions, and breeding experiments to attempt to increase parasite efficiency. Some parasites and predators serve as vectors of pathogens and might be useful to increase the incidence and spread of gypsy moth diseases. Several species of parasites have been released in large numbers to test the effectiveness of mass releases as a means of reducing host numbers in sparse and moderate populations of gypsy moth. In Europe, in periods between outbreaks when gypsy moth numbers are low and parasites would normally disperse, egg clusters have been added to increase the density of hosts and to retain and build parasite populations. Providing nesting boxes for insectivorous birds and creating or maintaining habitats favorable for small rodent populations could increase populations of larval and pupal predators.

The extensive research on pathogens has resulted in the registration of NPV as a biological pesticide. The lack of deleterious environmental effects and the self-perpetuating potential of the virus greatly increases its value as a pest management tool.

Disparlure

Disparlure, the synthesized sex pheromone, is the most useful tool for gypsy moth surveys, when used in traps to attract and catch males. Recent discovery that the + enantiomer of disparlure is most attractive to male moths has greatly increased the value of the attractant for surveys and as a gypsy moth control, by luring males from the population through use of disparlure-baited traps and by permeating the environment with pheromone to confuse the males and reduce their ability to find females. To be effective, the air permeation or confusion technique must reduce mating by at least 90 percent. The use of this technique appears to be limited to low populations of the gypsy moth. The reason for this is that disparlure elicits a searching response in males, with moths orienting to and flying up and down tree trunks. At distances of 0.3 to 0.6 m, males may utilize vision to find females; if females are numerous they may be seen and fertilized by males. In sparse populations, the chances of males seeing females is greatly reduced and the female pheromone trails will be masked by the pheromone permeating the atmosphere.

Genetic Control

There is renewed interest in the release of sterile males in natural populations as gypsy moth control. Most gypsy moth females mate only once, and the eggs of a female mated by a sterile male will not hatch. Sterile-male technique requires the ratio of sterilized to natural males to be about 100 to 1. Because of the numbers of sterilized males that would be required for moderate to heavy gypsy moth populations, the technique appears to be feasible only for sparse populations. Rearing costs and production of males of equal competitive ability as the males in the field are important considerations.

The extensive genetic studies by Richard Goldschmidt conducted earlier in this century showed that male gypsy moths from Japan, when crossed with gypsy moth females from Europe and North America, produced female progeny that were intersexes, which have characteristics of both sexes and are sterile. The release of male Japanese gypsy moths into North American gypsy moth populations has been suggested as a means of genetic control. Although numbers would be reduced initially because females produced from the crosses would be sterile, the hybrid males are fertile. When these males mate with North American gypsy moth females, only one-half of the resulting female progeny are intersexes; the remainder are fertile. This approach, therefore, would add the genes of the species from Japan into the North American gypsy moth population. When one considers that the Japanese species is considerably larger, and hybrid vigor or heterosis would most likely

result, the employment of this approach might compound the problem rather than alleviate it.

Silvicultural Control

Altering the forest composition to reduce the percentage of oaks to about 15 to 25 percent of the dominant and codominant trees will make the forest less susceptible to gypsy moth defoliation. In many of the eastern forests, oaks predominate, often utilizing sites not favorable for growth of many other tree species. In some areas, oaks are the most valuable trees for forest products. The removal of oaks, prevention of their regeneration, and encouragement of suitable tree species that might grow on these sites, plus the expense of these alterations, limit the use of silvicultural control, although in some instances this approach might be considered. Areas being developed for recreational use or for homesites often require the removal of trees, and oaks could be eliminated and less favored hosts, such as maples, retained.

Integrated Pest Management

Integrated pest management approaches, which involve the utilization of the proper control procedures to fit a particular situation, require a knowledge of the biotic and abiotic factors affecting the dynamics of populations and a reasonably accurate method of predicting population trends; adequate pest management tools and procedures to reduce population levels; careful monitoring of the pest populations; and a system under which management procedures can be implemented on the basis of recommendations of pest management specialists.

Few insects have been studied as much as the gypsy moth. Most of the factors associated with changes in population numbers have been identified, and a predictive model for assessing population trends has been developed. Adequate measures for population management are now available and are discussed in detail in this book.

The key to effective pest management for the gypsy moth will be how well the populations are monitored and the willingness of those controlling the resources—both forests and money—to respond. The tendency has been to respond to crises rather than to prevent them, but the continuation of such a policy will not result in an integrated pest management program. The alternatives are to do nothing, or to continue the firefighting approach, which has not been very effective. Once outbreaks develop, they spread quickly and greatly increase the area of the infestation. To reduce the numbers of gypsy moth to levels where the amount of defoliation is retarded, high numbers of larvae must be killed, limiting the control option to insecticides. The insecticides are applied after dispersal of first-instar larvae has occurred, and adjacent areas often need treatment in the following years.

Population monitoring could be restricted to sites favorable for buildup of gypsy moth populations. Monitoring would have to be done periodically each year, not just in those years when populations are high, and this would require maintaining a cadre of trained personnel.

The gypsy moth is unaware of political boundaries, but political decisions will be required at local, State and Federal levels when many of the pest management approaches are utilized. For integrated pest management to work, there must be a trust in the judgment of the pest management specialist and a positive response when resources are required to reduce gypsy moth numbers. Pest management procedures will often be required before there is visible evidence of defoliation. A reticence to respond in such situations will negate the resources and efforts devoted to developing an integrated pest management program.

Summary

What does the future hold for the gypsy moth in North America? Perhaps the future may be predicted by the past record. The gypsy moth has been in much of New England for 70 years or more. Historical accounts provide good documentation of its spread and damage. For example, the gypsy moth spread

into Maine about the turn of the century, and the amount of defoliation and area defoliated was extensive for about a decade. Since the first outbreak, the record shows a decline both in the number of hectares defoliated and the duration of each succeeding outbreak. The insect now appears to be acting more as a native insect pest than as an invading species. Although less dramatic, the same trend can be seen in the remaining New England States. One can only speculate on the reasons: The introduction and establishment of parasites and predators; the appearance of the virulent NPV, perhaps introduced in early shipments of parasites; a change in composition of the forests as some oaks were replaced with species less favorable to the gypsy moth; and genetic changes in the insect. It is likely that what has occurred in New England will occur in other regions that the gypsy moth invades. The stabilization process of populations will unfortunately not be rapid if allowed to progress naturally and underlines the necessity of developing sound pest management strategies.

Methods of Gypsy Moth Detection and Evaluation

<div style="text-align:right">**3**</div>

Introduction

Robert L. Talerico

Detection and evaluation of the gypsy moth/host population interaction form the core of any pest management or decisionmaking procedure. Detection and evaluation methods rely upon sound, detailed knowledge of pest/host biological and ecological relationships; this knowledge, when coupled with economic or sociological data, allows decisions regarding management alternatives to be made on a rational basis.

Techniques of monitoring and assessing the gypsy moth and the understanding of the overall effects of the damage it causes have improved greatly in the past few years, but suitable operational methods to quantify many of the ecological, economic, and sociological impacts are still lacking. Furthermore, aesthetic, moral, political, and emotional impacts evade measurement. Impacts of this kind, labeled axiological (Stark 1977), add another dimension to measurement problems, but they also provide a location for the effects that appear imponderable and are not ecological or that cannot be judged on economic terms. When quantification of these effects is attempted, it is apparent that many economists disregard ecology and aesthetics, while many ecologists will not confront economic reality.

Detecting Populations

Robert L. Talerico

High gypsy moth populations are not difficult to find; such populations announce their presence through larval activity and by defoliation visible by air or from the ground. However, if a pest management program is to be effective in minimizing damage, detection should be possible when insect density is very low, scattered in clumps or pockets and building in magnitude. During this phase, treatment is simpler and easier because it is confined to a small area, which minimizes environmental pollution, and because a variety of pest management alternatives may still be considered and implemented. Detection of low-level gypsy moth populations is also useful and desirable because it eliminates any sudden, unannounced

eruptions of the insect, provides information on rate and direction of the population movement, and permits lead time for planning and scheduling of ground surveys, hazard rating of stands, control treatments, or salvage operations. More importantly, it provides lead time for managers to plan and budget the resources necessary to carry out future intervention.

Egg Masses

The current method of egg-mass detection is by eye; a means of detecting egg masses that does not rely upon visual inspection would be useful in research, regulatory, and control programs. Since most organisms emit odors, which are considered chemical messengers, their detection would reveal some stage of the organism. Unfortunately, these odors are often released at very low emission rates, making odor detection very difficult, especially for humans. However, man has successfully utilized domestic canines for hunting and tracking of various biological organisms, and these same animals have been trained to detect inanimate objects such as drugs and explosives.

Wallner and Ellis (1976) successfully trained three German shepherds to detect and locate gypsy moth egg masses by their odor. The shepherds were able to detect egg masses from as far away as 2 m, which suggests that these animals could be used for quarantine inspection of vehicles or to detect suspected infestations of the gypsy moth. Further tests could be employed to estimate egg-mass density by relating the number found in a prescribed search period to actual field density.

Adults

Forms of chemical communication between biological organisms have been investigated intensively during the past 10 to 20 years. The isolation and chemical characterization of a sex pheromone or attractant for the gypsy moth have been difficult processes. Forbush and Fernald (1896) were aware that the female gypsy moth was able to attract males from various distances up to 0.8 km and female-baited traps were used

in early survey work. Difficulties with the use of live females led to the development of extracts from the female's abdomen and finally to the characterization of the active component of the attractant. A detailed and complete history of the development of the gypsy moth sex attractant is provided in the section on pheromones (6.4).

The use of the gypsy moth sex attractant or pheromone for survey purposes has been detailed in an agency operating manual for gypsy moth surveys (U.S. Department of Agriculture APHIS 1977). Specific information is presented on types of surveys using the pheromone, timing of the surveys, distance between trap locations, and training information for trap-tending personnel that includes trap site selection, trap design, and placement methods. These methods have been extracted from a variety of sources and assembled into a workable set of guidelines for the field worker.

Quarantine Areas

Spread of the gypsy moth occurs in two ways: By windblown dispersal of the newly hatched larvae, and by the inadvertent transport of the insect—primarily egg masses—attached to vehicles, building material, and almost any other movable object. Wind dispersal results in local spread or movement; movement on manmade objects causes long-distance movement.

Forbush and Fernald (1896) provide an excellent account of the dispersal of gypsy moth larvae moved by wagons from infested areas. By 1905, the infestation had spread from the initial infestation north of Boston to Maine, New Hampshire, and Rhode Island. An effort was made to prevent the shipment of infested products into outlying areas, an action that eventually lead to Congressional passage of the Plant Quarantine Act of 1912.

From this act, a cooperative gypsy moth regulatory control and containment program evolved. This program is a joint planning and financial undertaking with the concerned States. Interstate movement of commodities that may be infested with this insect are regulated by a Federal quarantine (fig. 3-1). Intrastate movement of these materials is regulated under parallel State quarantines.

Inspection and control procedures for enforcing the Federal quarantine regulations are available from the Plant Protection and Quarantine Division of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (APHIS) (1976). These procedures provide State and Federal inspectors with detailed guidelines on how to examine various commodities for the life stages of the gypsy moth. If an infestation is detected, procedures are described for treatment before the shipment is approved for movement beyond the quarantine area. Monitoring this type of shipment is relatively simple.

The greatest concern is with the movement of recreational vehicles and mobile homes from the generally infested northeastern region southward and to the West Coast, which has resulted in spot infestations along the most frequently traveled routes. Unfortunately, inspecting such vehicles in transit would be time consuming and expensive.

Evaluating Populations
Robert L. Talerico

States within and at the periphery of the area generally infested by the gypsy moth conduct annual surveys to determine where the gypsy moth is located, assess density, and speculate on defoliation prospects for the coming year. Aerial and ground defoliation surveys and fall and winter egg-mass surveys are used to develop a composite picture of the situation. This information is then used to revise records on gypsy moth spread for regulatory activities and to plan suppression programs. State and Federal agencies cooperatively collect and share this information. Of course, insect density is only one of the items that should be considered in the process of deciding if an area should be scheduled for control. Other important factors include:

- Egg-mass size.
- Parasitism and predation.
- Evidence of virus.
- Length and intensity of outbreak.

Legend:
- Area subject to quarantine regulation
- Counties where male moths were detected

Washington State

Scale
0   100   200 mi.
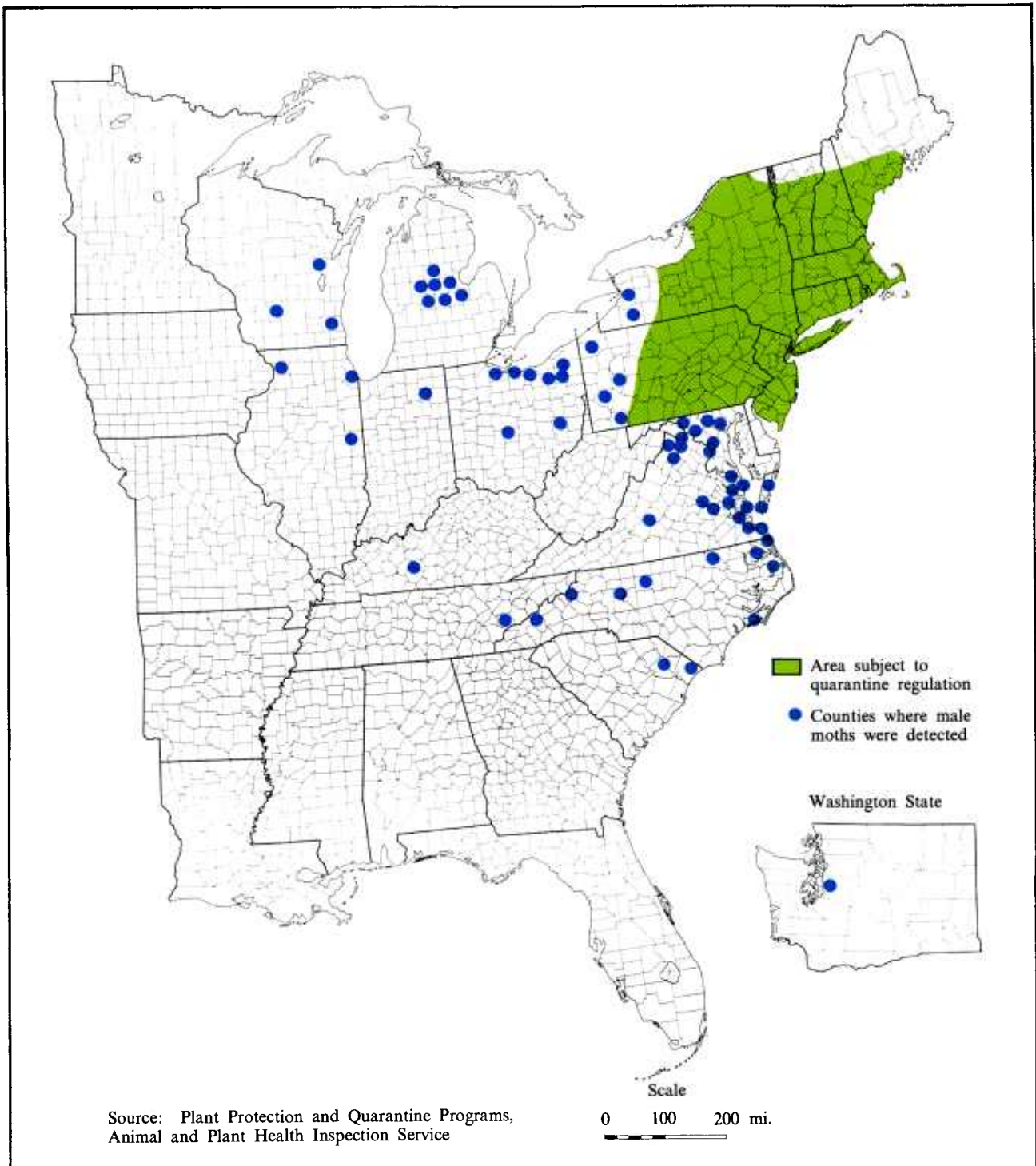
Figure 3–1.—*Gypsy moth quarantine areas, 1978.*

- Overwintering gypsy moth mortality.
- Forest tree species composition.
- Tree condition, tree size, and stocking density.
- Forest use.
- Water hazards.
- Weather.

Over the years, rule-of-thumb guidelines have evolved and been incorporated into state and Federal operating plans. These guidelines relate broad estimates of egg-mass numbers per hectare to broad categories of expected defoliation. Most operational programs use a criterion for treatment of 1,236 egg masses per hectare. This threshold is believed to indicate the potential for heavy defoliation the following season. Before decisions are finalized, this egg-mass density value is weighed in relation to the above factors.

Some areas of high use or valuable timber that have egg-mass counts in the range of 741 to 1,236 egg masses per hectare may be proposed for treatment because of their proximity to very heavy gyspy moth populations. The treatment is to provide protection from windblown larvae and large larval migration. Other States prefer to use a minimum threshold of 618 per hectare as their requirement for treatment. These differences reflect various experiences and backgrounds with the gypsy moth over the years.

There are additional uses made of egg-mass density measurements. Estimates made on the residual egg-mass population following treatment are one of the gauges used to measure the effectiveness of a treatment program. Another use is for monitoring population change. Estimates made regularly over time provide an index of population trend, which is useful for speculating on what conditions might be in the future, keeping abreast of potential gypsy moth problem areas, and planning surveys.

Direct Evaluation
Robert W. Wilson, Stephen M. Ivanowsky, and Robert L. Talerico

Any insect stage could be used to determine gypsy moth population density; however, larvae, pupae, and adults are present for short periods and would present some difficult sampling problems. The egg mass is favored because it is stable over time and available for at least 8 months, thus provides adequate time for obtaining population estimates and planning a control program if needed.

The complete examination of the susceptible area was replaced with observations on small plots scattered through the area. This was a much more efficient method, but it relied heavily on the training, experience, and thoroughness of the observer. Undetected or missed egg masses became a critical sampling problem, especially in borderline cases.

The method employed by New York is representative of methods used in the region (New York State Conservation Department 1963). Ground surveys were made of susceptible areas by using a line strip plot 80.5 m long and 10 m wide. Live trees, dead and down trees, undergrowth, rocks, and litter were observed for egg masses only in the direction of travel, so the effective area observed was 0.04 hectare. Plot length was determined by pacing, and width was judged by eye. The number of plots to establish varied by size of area and experience of the observer. A plot could be observed, on the average, in 4 minutes. An additional 2 minutes were added if light colored bark (white and gray birch) or loose bark trees (white oak) were in abundance. These background bark conditions make detection of egg masses more difficult.

When the count of visible egg masses was completed, a prepared list was examined to provide correction factors for undetected egg masses. The list contained a number of factors that could influence egg-mass abundance, such as tree cavities, litter, stone walls, fences, loose bark, and snow depth. Each had a numerical score; the scores that best described the area were totaled. This total was then used to find the appropriate inflation factor from categories that bracketed three condition classes with numerical values of 3, 4, or 5, 5 being the most severe. The class value was used as a multiplier for the observed egg-mass count and the result converted to 0.4 ha values.

A later revision of this method eliminated the need to score and total the individual factor that could affect abundance. Instead, the 3, 4, and 5 multipliers were used to represent forest conditions from open with a few trees (3) to densely wooded (5).

Different size sampling plots without correction factors are used by others. Some State organizations and researchers use 0.04-ha or 0.01-ha plots that may be circular or square. New Jersey uses a more rigid system to examine egg-mass density. Observers count egg masses on 0.4-ha plots every 0.3 km on a grid system that encompasses the area of known infestation. Users of line strip plots feel that this method is less time consuming because time is not spent measuring boundaries. All of these plot sizes, however, contain considerable surface area or locations for egg masses—live trees, dead and down trees, branches, undergrowth, rocks, litter, and so forth. To thoroughly examine all locations in even a 0.04-ha plot with some degree of certainty would require a sizable investment in time and manpower. A series of smaller plots scattered over the area can provide a much better indication of conditions and a better balance between the certainty of locating egg masses and cost.

In 1973, workers in New York State (New York State Department of Environmental Conservation 1973) began to use a prism-point sampling method to estimate egg-mass density. Prism-point sampling has been used in forestry for many years, and the theory and procedures are well documented. This method uses the prism to select the live trees to observe for egg masses. Tables and formulas were developed to provide estimates of egg-mass numbers per hectare and workers feel the method is much quicker and yields more reliable results than the line strip plot method.

Egg-Mass Numbers and Quality

Fixed- and Variable-Radius Plot (*FVP*) Method

Detection of gypsy moth egg masses in sparse or low-density populations is difficult because a great majority of the egg masses are deposited on the tree near the ground, in the litter, or in protected locations. Concurrently, but independently of New York State, in 1974 the Forest Service began to investigate the use of prism-point sampling for observing gypsy moth egg masses (Wilson and Fontaine 1978). This method used the prism method to select the live trees for observing egg masses but included a fixed-radius, 0.002-ha plot for observing egg masses on all other material.

In selecting this type of sampling plan, the following factors were considered:

• Use of small sampling units requires a limited attention span for the observer, which encourages careful and accurate counts by field personnel. In larger units, (0.04 to 0.4 ha), the observer cannot examine the area in detail in the time he feels should be allotted to the survey. This fosters a cursory examination contrary to the survey objectives. Smaller units give the feeling of accomplishment, permit the progressive movement through an area, and should improve the reliability of the survey data.

• Although variation among small sampling units is greater than among larger ones, the cost is proportionally less. Thus, larger sample sizes can be drawn from sampling distributions that tend toward normality, even with a strongly aggregated underlying egg-mass distribution. This simultaneously maintains a given level of precision.

• Sampling effort is concentrated in the largest component of the egg-mass population. Investigations in central Pennsylvania showed that 85 percent of the total number of egg masses per 0.04 ha were located on the live overstory trees and varied in proportion to tree size.

• The methods are simple and straightforward for field use and can be used for other forest assessment problems such as defoliation estimation for large areas. The necessary computation can be done on a pocket calculator.

The sample selection procedure is critical to the success of the survey. A simple probability selection scheme meets the needs of simplicity in application, reasonable cost, and an unbiased estimate of the items

of interest. This plan employs systematic sampling with a single random start. A uniformly spaced grid is superimposed on a map of the area to be sampled. Each point is a center for a fixed-radius ground plot to search for egg masses and a prism point for selecting live overstory trees to observe for egg masses.

The number of points (sample size) to use depends upon the precision required in the egg-mass density estimate and on the inherent variability in the egg-mass population of interest. Variability in the population is, in turn, dependent on the egg-mass density. It is possible to sidestep these questions by accepting a sampling error of 20 percent of the mean density when that mean is about 741 egg masses per hectare. Under these conditions, 30 sampling points are required, a number that will also give reasonable precision at other egg-mass densities. Alternatively, provisions are also made for calculating sample size for any combination of sampling error, confidence level, and anticipated egg-mass density.

A field crew of three people is preferred to handle the multiplicity of jobs and speed the work. All crew members should be adequately trained in all procedures so that work responsibilities can be rotated. This reduces errors resulting from monotony and job fatigue.

Egg-mass surveys can begin immediately after moth flight has ceased, effectively extending the period of counting egg masses by 2 months. Waiting until leaf fall was found to have no appreciable effect on the ability to observe egg masses. By starting earlier, more time is available for conducting the survey, and additional reevaluation of questionable areas is possible. Finally, more time is available for the overall planning of any treatment.

This method was developed for areas of 4.1 ha or more and where egg-mass density is in excess of 247 egg masses per hectare. Searching for egg masses at low densities is usually unreliable, inefficient, and costly. Use of the sex pheromone disparlure is expected to be more efficient for these conditions.

Five-Minute Walks for Observing Egg Masses

A rapid procedure for determining the magnitude or index of the gypsy moth egg population is a handy tool for the field worker. Such a method would be useful for individuals planning control programs to assess rapidly proposed areas for the need of a more intensive sampling (FVP) or to monitor population trends over time.

This method is easy to use. A two-man crew is deployed in single file. Both count visible egg masses, but the first individual's primary responsibility is to serve as a guide for the second, who follows behind concentrating on observing and counting egg masses. Direction of travel and movement is not restricted. Either individual can keep track of time. When 5 minutes have elapsed, the observers total their counts of egg masses and divide by two. Two to three counts by this method should be adequate to classify an area. A regression relationship has been developed to relate the 5-minute counts to counts obtained by the FVP method (fig. 3–2). The coefficient of determination (R^2) for this relationship is 0.83.

Actual vs. Observed Egg Masses

Visual egg-mass observations are plagued by the problem of reliability. Regardless of how thorough the search, egg masses are overlooked. Many factors can influence the observation process: weather conditions, type of ground litter, tree species, stand density, tree height, infestation level, presence of old and new egg masses, and observer experience.

The central Pennsylvania infestation provided an opportunity to examine this problem on selected trees of white oak (41 trees) and associated oak species (27 trees). Visual observations of all trees were made in late August and again in mid-October by individuals with experience ranging from none to over 4 years. Two observation periods were included to examine the repeatability of an individual's egg-mass counts and to pinpoint the sources of variability between the two observed counts. The observers were allowed 1 full day to count the egg masses. Observation time averaged about 10 minutes per tree. Binoculars were the only aid permitted. After the second observation, the trees were cut and the bole and large branches examined in detail for egg masses. Most likely a few egg masses were destroyed in the felling process, but the number was probably insignificant.
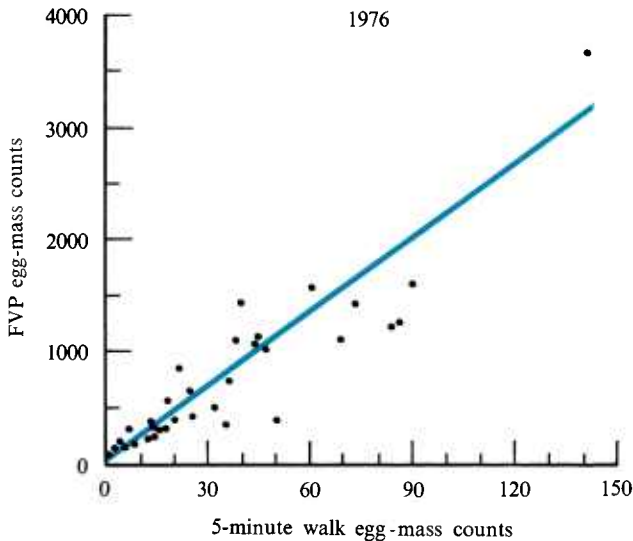
Figure 3–2.—*Relationship between 5-minute walks and FVP egg-mass counts.*

A preliminary analysis comparing actual and observed egg-mass counts tree by tree revealed that about half of the egg masses on white oak were not counted, but very few were missed on other oak species. There were also differences in count accuracy among observers, but these were unrelated to experience except that first-time observers missed more egg masses on white oaks than experienced observers. A more detailed analysis of main effects and interactions is being made, and a method will be developed to deal with the troublesome white oak egg-mass counts.

Estimating Population Quality

The quality of the insects comprising a population may be a significant factor in the success of that population. For the gypsy moth, qualitative changes usually occur in response to increasing density. This appears to be characterized by a nutritive change affecting the physiology of the insect (Leonard 1971). By analyzing egg-mass characteristics, rearing larvae from these masses, and observing food sources in the area, information on population quality can be derived. This information can be used to guide population assessment and control recommendations. If a gypsy moth population is judged

"unhealthy," control would be unnecessary and result in a savings of time and money.

Various symptoms of "unhealthy" populations have surfaced through observations and experiments. Egg-mass size and number of eggs per mass have been found to correlate with population change (Richerson et al. 1978). New developing infestations have a greater number of eggs per mass than older populations, and the masses are visibly larger. These populations also have a greater number of fertile nonparasitized eggs per mass and a higher rate of successful hatching.

Although costly and time consuming, rearing of the larvae from these egg masses will also provide additional information about the population. Additional molts might be detected that could signal nutritional problems (Leonard 1971). More significantly, the rearing will indicate the degree of latent NPV infection in the population. Should it be high, treatment might be unnecessary, provided a certain amount of defoliation can be tolerated.

The food sources present at a location also have a significant influence on the resulting population (Capinera and Barbosa 1977). Preferred food (oak) produces larger pupae and highly fecund adults, which in turn produce larger eggs. On the other hand, maple diets are deleterious to the gypsy moth, producing smaller pupae, lower fecundity, and smaller eggs. An oak beech diet appears to produce the same effects as maple, while older instars feeding on white pine react as those feeding on oak.

An important factor in the population dynamics of the gypsy moth is variation in the sex ratio. A method to determine this ratio in the population at the beginning of the larval stage and at later stages is desirable. A whole-mount microscope technique has been developed to determine the sex of fully developed embryos and early-instar larvae (Levesque 1963). Proper staining technique and magnified observation of the gonads make this determination relatively simple and precise.

An evaluation of population quality would add another expense to the cost of current methods of population assessment for control purposes. However, an intensive examination of gypsy moth egg

masses, larvae, and hosts would provide more information for the decisionmaker. The collection of egg masses and information on hosts could easily be worked into the FVP sampling method.

Larvae and Adults

Suppression efforts are usually evaluated on the basis of reduction in the egg-mass population after treatment. This reliance on only one measure could lead to an erroneous conclusion about the treatment. For instance, in a treated area, gypsy moth larvae might die from causes other than the treatment— parasites, predators, or virus, for example. Unknowingly, this mortality would be attributed to the treatment. If some intermediate measures of the population were available, these would supplement the final egg-mass survey and confirm or deny the effects of the treatment. Intermediate measures of population change are especially important when assessing the effects of microbials and the newer growth-regulating chemicals. The action of these materials is not as rapid as conventional chemicals, and this increases the opportunity for other factors to interfere with or alter the results of the treatment.

Sampling gypsy moth larval populations is a difficult problem because of their location and movement. Several indirect methods have been proposed for use. One method uses 10-minute walks to observe and count all visible larvae on tree trunks, branches, foliage, understory vegetation, litter, etc. (Connola et al. 1966). Because larval activity is affected by weather conditions, counts tend to be lower on overcast days compared to bright, sunny days. This information should be recorded with the observed larval count. These counts can be used when third-instar larvae are present and continue to pupation.

Other techniques for sampling pretreatment and posttreatment larval populations have been used. Doane and Schaefer (1971) made pretreatment counts of the number of larvae per twig terminal. The twig terminals were selected at random as each observer moved through the woods. Similar counts were made in treated and untreated plots.

The same workers made posttreatment counts of the number of living larvae on 60-cm branch terminals and on 60-cm sections of oak trunks using one-half the circumference of the trunk at about chest height. The branch terminals and trees were selected at random as the observer moved through the woods.

Frass collections can also be used to monitor larval activity (Connola et al. 1966). Frass can be collected for various time periods to provide an index of the population. Connola et al. (1966) describe the use of 1×2 m fine mesh cheesecloth drop cloths erected in hammock fashion to collect frass. The cloths are positioned directly beneath infested foliage. A small weight is placed in the center so that the frass will collect in the depression and not blow away. The dry frass is collected as often as possible but at least every 4 days. After collection, the frass is cleaned of debris— leaf fragments, cast skins, other frass, etc—and weighed. The frass can be screened to separate the various larval instars and to obtain frass production estimates by instar for each collection or production by day per drop cloth.

Frass collecting is prone to many disturbances. Wind and leaf movement can deflect frass from the drop cloth or blow more into the cloth. Condensation and rain make the frass difficult to collect and separate from other debris. Animals can also upset the cloths, resulting in breakage and loss of frass.

Adult activity can be monitored with the gypsy moth pheromone. Baited traps can be placed in areas of interest to learn if male moths are present. Once detected, delimiting the infestation boundaries requires more effort. This can be done effectively with a pheromone-baited trap, arranged in a grid pattern (see Disparlure-Baited Traps for Survey and Detection, chapter 6.4). In addition, the Plant Protection and Quarantine Division of the Animal and Plant Health Inspection Service is working on relating trap-catch densities to egg-mass numbers.

Defoliation as an Indirect Means of Population Assessment
Robert L. Talerico

Next to larval abundance, defoliation is the most obvious indication of a gypsy moth problem. Like vis-

ual observation of egg masses, defoliation estimating methods vary with individuals and by organizations. Rating schemes are usually timed to depict peak or maximum defoliation. The schemes used range from broad defoliation categories of light, moderate, or heavy, to the ranking of defoliation by decimal units expressed as a percentage. A combination of these two methods is also used—for instance, light defoliation might be equated to the decimal units of 0–30 percent.

All these categories are subjective, and anyone using them has his or her own mental impression of appearance. Although the human eye is a good integrator of the presence or absence of foliage, standards for defoliation categories of hardwood trees are not available for reference. Nevertheless, defoliated acreage by categories is used as one indication of the potential need for control projects the following year.

The visual rating of defoliation is also complicated by a variety of problems beyond the control of the observer. Observing a tree crown in a forest is difficult because of the surrounding trees, the shape of the crown, background lighting, sun angle, and viewing angle. Obviously, an open grown tree has a much different shape and quantity of foliage than one growing in competition with others in a forest situation. Crown shape also varies within and among tree species.

The ability to express defoliation levels accurately is needed if valuable trees or stands are to be kept in peak growing condition. Different levels of defoliation trigger specific responses by the tree. One level causes the tree to try to refoliate that season. A lower level elicits no response, while a greater degree results in no attempt to refoliate that season. These responses result from complicated physiological processes that are tempered by many biological and physical factors or processes not yet completely understood. Refoliation causes a depletion in food reserves of the trees that could affect tree conditions in subsequent years. If the critical level of defoliation was known and the refoliation process understood, treatment could be targeted to prevent excessive defoliation and to limit such responses as tree and branch mortality and reduced increment.

Ground

Visual

Visual ground estimates of defoliation are made to classify a particular location or tree. This might be done to determine infested areas, rate locations for planning control projects, or determine the protection received from a control treatment. If large areas are to be surveyed, a large, trained, experienced work force would be needed to cover the area during peak defoliation. The interactions among weather, insects and trees cause the time of peak defoliation to vary by location, larval development, and stand composition from June 15 to July 15 for the gypsy moth.

Details about observing defoliation on hardwoods are few. The simplest advice is to observe tree-crown foliage with or without binoculars and to estimate the percent of foliage removed (Connola et al. 1966). Another method is to observe sample leaves selected at random for various parts of the crown and to estimate the average percent of leaf surface removed. Some individuals rate preferred hosts (oak) separately and then make an estimate of the forest canopy in general.

Point Sampling

At times, estimates of defoliation are needed for a large area to record foliage protection provided by a control measure, to monitor defoliation for a specific research project, or for other reasons. The FVP method can be used to acquire these estimates.

For this use, the FVP plot layout should be established in the same way as when observing egg masses, but because the low vegetation, litter, etc., are not rated for defoliation, the fixed plot is eliminated. The sample point is located and the same prism is used to select trees for observing defoliation. The tree is rated by estimating the amount of foliage removed and record is kept for each tree by sample plot. When all points have been observed, the defoliation rankings are totaled and divided by the number of plots to obtain an area estimate of defoliation. Ancillary information can also be recorded if desired. Defoliation rankings can be recorded by tree diameter, species, or crown class, and estimates can be

made on this basis. The result is a ground estimate of defoliation that is representative of the area of interest.

Aerial

Aerial methods for observing defoliation provide a synoptic view of conditions over a large area. Data from an aerial survey are obtained rapidly, provide a timely picture of conditions, and, with proper planning, can be very thorough. Weather conditions and haze can interfere with the timing of flights, but this should not be a major problem. Several methods of aerial observation are used. Of course, all methods should be supported by ground information to verify the cause of defoliation and the intensity.

Sketch Mapping

Aerial sketch mapping is conducted as a line strip plot ground survey except that 100 percent coverage is necessary for the entire area. Flight lines are usually spaced 6.5 km apart, the distance normally seen by observers flying at air speeds of 140 to 160 km per hour and at altitudes of 610 to 914 km. Two observers are used, each observing a portion of the flight line from one side of the plane. A third crew member is frequently used as a navigator or a tracker to assist the pilot in getting on and maintaining the flight line.

Aerial sketch mapping is done on large-scale maps that are bulky and difficult to handle in the small cabin of an airplane. To overcome this difficulty, a map-rolling device is used (Merkel et al. 1955) that permits a long strip of maps to be contained in a very small space and provides a sketching surface for the observer.

A variety of maps can be used as a base. County highway, National Forest, or U.S. Geological Survey topographic maps have been used successfully. Maps to be used in the survey are assembled and glued together. Flight lines are added and the map cut into strips that may cover one or more flight lines. The strips are taped together in sequence and wound in the map roller.

In operation, the observer holds the device in his or her lap and advances the maps as the terrain passes below. Infested areas are plotted as observed. Later on, the ground observers transfer their observations to a map of the whole area and reconcile defoliation boundary differences. Areas in each category can be determined with a planimeter or dot grid.

Operations Recorder

If greater precision is necessary, the same type of survey can be conducted with an operations recorder (Heller et al. 1952). Greater control must be maintained over navigation, height, and speed of aircraft and data recorded. In order to obtain a fivechain (100.6 m) strip, the airplane must be flown at 152.4 m above the ground, and speed must remain constant between check points. The observers record timber type —nonforested, pine, hardwood, etc., and degree of defoliation along the flight line with an operations recorder. Ground checking should be done to verify the aerial estimates of forest type and defoliation. Analysis is by flight lines and means and variances can be calculated. The primary limitations of this technique are that it is restricted to flat terrain and is most efficient for large areas.

Published data on areas defoliated by the gypsy moth in various States are confusing and difficult to interpret, because no standard categories of defoliation have been adopted, and until recently, no practical means for differentiating degrees of defoliation were available (Talerico et al. 1977). As a result, defoliation just barely visible in an aerial survey in one State may be classified as light; in another, such as Pennsylvania, it may be classified as moderate because in their system, light defoliation is considered to be detectable only from the ground. Pennsylvania reports no light defoliation because it does not conduct the ground surveys needed to delineate this level. Yet this level of defoliation, which probably affects thousands of acres of Pennsylvania forests each year, is not reported.

Other problems also influence the reliability of these surveys. The defoliation estimates rely upon the observer's experience, motivation, and subjective judgement of the amount of foliage present or absent.

During the flight, the observer must contend with many personal and physical problems that can instantaneous while flying at 140 to 160 km per hour. Other typical problems are long periods of flying in a cramped position which affects the attention span, boredom when damage is not evident, sun angle, and even air sickness with certain flight conditions.

Aerial Photographs

Aerial photography offers a means to permanently record forest canopy conditions on a large area very rapidly and with considerable detail. This method eliminates many of the personal comfort and physical problems encountered in sketch mapping, but it is still weather dependent and relies upon subjective classification on defoliation categories. Classification by the photo interpreter depends upon color, tone, brightness, and texture differences. The interpreter critically examines suspect areas in detail and, where necessary, uses optical magnification before a decision is made on the defoliation level. Over time, an interpreter can develop reference material to document various levels of defoliation. The reference material should be supported with ground observations.

Film type, scale, and timing of the photography affect the information that can be extracted from the film. Kodak® Aerochrome MS film 2448 (TC) and Kodak® Aerochrome Infrared film (CIR) 2443 are frequently used to record defoliation. CIR film has an advantage because of its ability to enhance subtle differences in reflectance, which are barely discernible in the visible wavelengths alone, and because of its haze-penetration characteristics. This film also records near-infrared and red-energy relationships, which have been shown to indicate vegetation stress (Colwell 1956).

The scale of the photography influences the amount of detail that can be seen on the film. If individual tree crowns or small plots are to be observed, a large scale is indicated, but if large forested areas are to be observed, a moderate to small scale is appropriate. The scale of the photography can greatly influence costs and must be balanced against the objective of the photography.

Timing of photography is important because foliage is developing and maturing as the insects feed. As the larvae grow, the rate of feeding increases, and large larvae are responsible for most of the defoliation. Moreover, following larval feeding, trees may add new foliage. If photographs are acquired too soon or too late, an erroneous view of defoliation will result.

Photometric Interpretation

Aerial photographs are a unique method for viewing defoliation because they provide a visual record of how the forest canopy appeared at a particular point in time. Sequential photography of an area can be examined and qualitative comparisons of the defolited areas made within or between years. However, peak defoliation does not occur uniformly over a large, hilly, forested area. As a result, this type of comparison is subjective and influenced by many factors that interact to affect the final film image— atmospheric conditions, camera and lens factors, film handling before and after photography, and film processing. The role of peripheral effects on film analysis are varied and complex (Lillesand 1976), but a relatively new technique is available to correct for these variables and permit accurate objective sequential comparisons.

Methods to quantify and negate peripheral effects have been developed and described in terms of photometric interpretation (Piech and Walker 1971, 1972). This process originally evolved from intensive efforts to expedite the detection of stressed vegetation using aerial photographs. For photometric interpretation, the camera and photograph become a precision photometer enabling measurements of ground reflectance over large areas. Through ground truth, reflectance measures can be related to a ground variable of interest—defoliation. This method should reduce the field data-collection effort dramatically.

The scene color standard (SCS) technique (Peich and Walker 1971, 1972) involves a camera photometric calibration technique and a new information extraction system, photometric interpretation, used for analyzing the color aerial film. Photometric interpretation is an analytical method in which the apparent densities of objects, including the atmosphere, flare light, and the recording system, are removed to provide true reflectance values of ground objects. Lillesand (1976) provides a detailed description of this method.

The SCS technique was used to develop a photometric measure of defoliation for CIR film at a scale of 1:31,640, which was related to visual estimates of defoliation from the ground (fig. 3–3). The third-order polynomial regression equation that describes this relationship is:

$$\text{Percent defoliation (CIR)} = [-475.0900 + 41.7470X - 0.9925X^2 + 0.0072X^3] \times 100$$

where

$$X = \frac{R_{IR}}{R_R} \times \frac{R_G}{R_{GM}} \times \frac{R_{IR} + R_R + R_G - 1}{3}$$

R_{IR} = crown reflectance in the near infrared.
R_R = crown reflectance in the red.
R_G = crown reflectance in the green.
R_{GM} = 0.03, crown reflectance in the green at maturity.

This polynomial provides a more reasonable description of the data at the extremes and a slightly higher correlation ($R^2 = 0.78$) than a linear model (Talerico et al. 1977). An examination of the residual sum of squares for each model indicated that the polynomial coefficients were adding significant information to the function.

For CIR film, this measure of defoliation is independent of photo scale. Limited use has revealed two problems. First, the location of the sun spot on the film has a significant effect on the measured reflectance values. This sun look angle relation has been termed perspective (shadow) projection (Walker
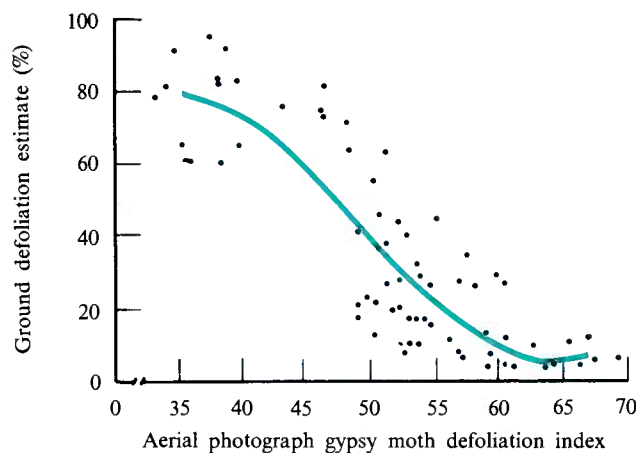
Figure 3–3.—*Relationship between photometric defoliation measured and observed defoliation.*

et al. 1977). Once identified, correction can easily be made for this factor. The second problem relates to crown condition at the time of measurement. The effective chlorophyll mass and/or leaf stacking of vigorous crowns appears to be greater than that of previously defoliated crowns. The photometric measure was developed from a canopy that had been defoliated or prestressed a year earlier, and as a result, the measure appears to be valid for prestressed crowns over the full range of 0 to 100 percent defoliation. But this relationship is valid for all crowns within the range of 30 to 100 percent, regardless of prestress. Since this is the range frequently mapped by aerial sketch mapping, the photometric method can be considered as good as aerial sketch mapping.

CIR photographs of the 1975 Bald Eagle State Forest test site in Pennsylvania, exposed on July 1, 1975, were assembled into a mosaic of the area (fig. 3–4). From this film a color-encoded infrared-to-red ratio mask was made and examined to determine how well this ratio depicted defoliation effect. The resulting map is shown in figure 3–5 and demonstrates the detail that can be achieved in comparison to an aerial sketch map of the same general area (fig. 3–6). If low levels of defoliation can be detected with these methods, infestation foci might be detected and marked for further monitoring or for early treatment.

Satellite

The repetitive coverage afforded by the NASA Landsat satellites makes this system ideal for monitoring and assessing defoliation over large geographic regions on an operational basis. The satellites are able to depict current conditions over large regions in a matter of hours, thus practically eliminating differences in conditions from one side of a region to another. The periodic coverage permits an interpreter to view the defoliation of a region from start to finish and construct a realistic defoliation map that accounts for topographic and insect host effects. Similar coverage with aerial photographs would require days or weeks with aerial sketch mapping; during this time tree condition would undergo

considerable change, and therefore the map would not reflect the true condition.

Like any aerial system, cloud cover limits collection efforts by Landsat. A recent examination of Landsat records for the gypsy moth infested area in the Northeast showed that cloud cover of 30 percent or greater is possible 60 percent of the time between June 15 and July 15, the approximate period of peak gypsy moth defoliation. During this time, special priority authorization for Landsat data may be necessary to assure availability of the imagery needed to generate a defoliation map.

Rhode and Moore (1974) showed that it is possible to assess gypsy moth defoliation from satellite imagery. However, they were not able to quantify degrees of defoliation accurately and relied upon
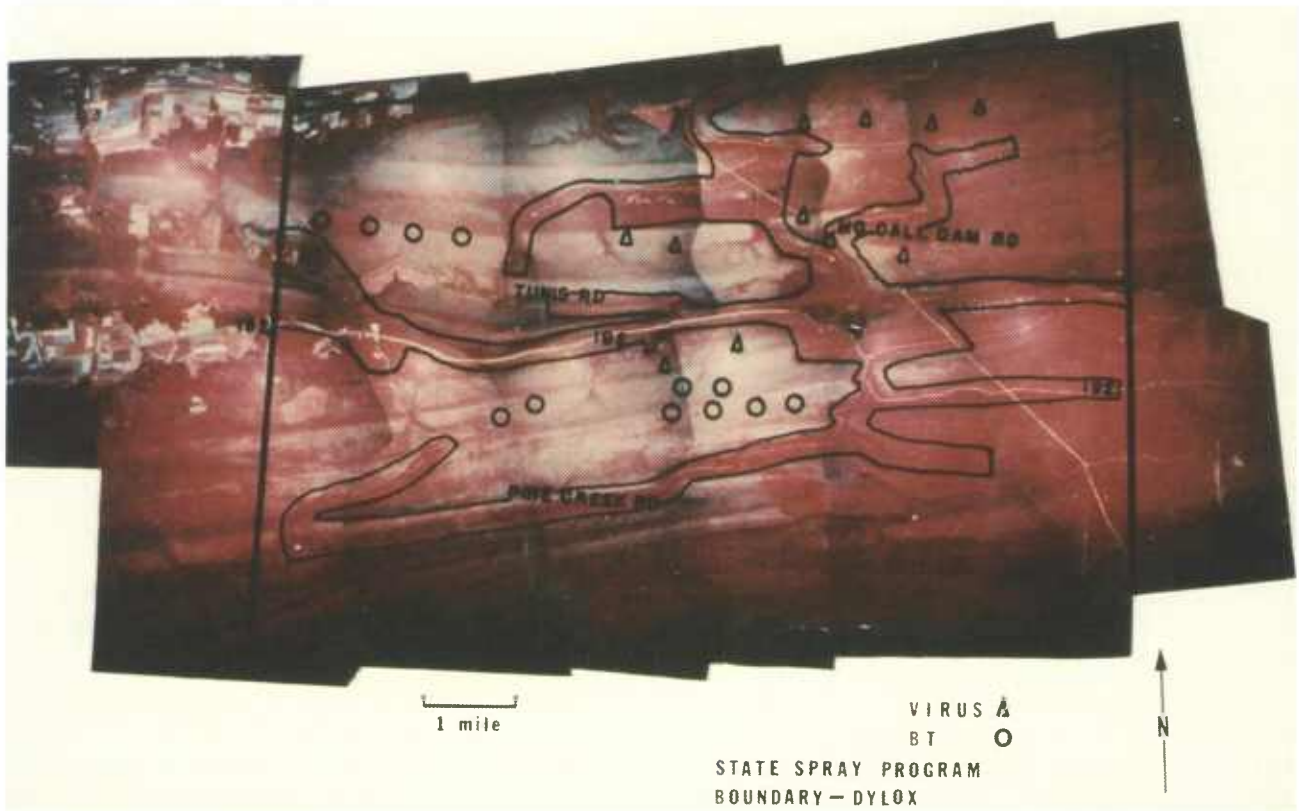
Figure 3–4.—*Color infrared mosaic of Bald Eagle State Forest, Pa., test site.*
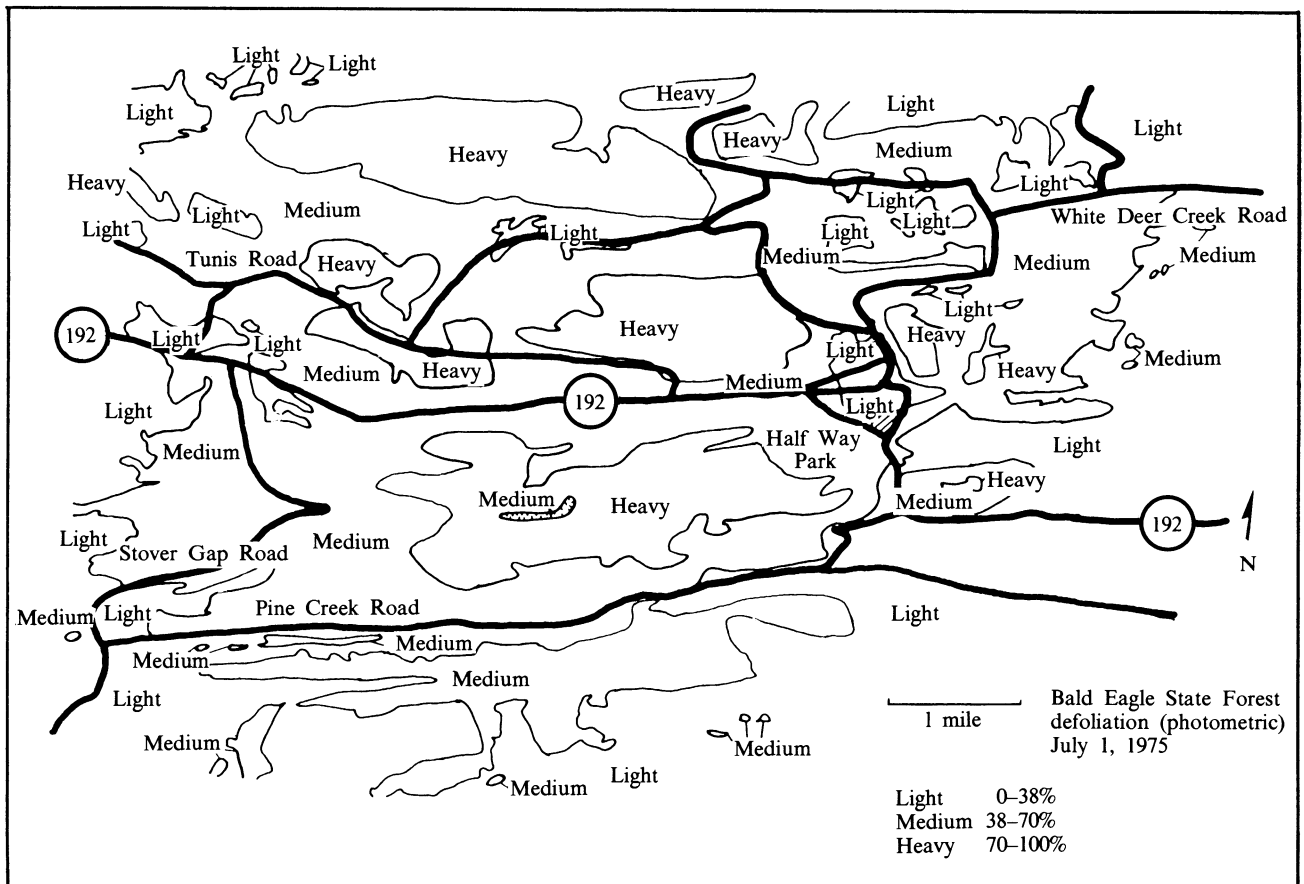
Figure 3-5.—*Photometric defoliation map from July 1, 1975, CIR film of test site.*

conventional photo interpretation clues such as uncalibrated brightness and tonal changes to distinguish among heavy defoliation, light-to-moderate defoliation, and no defoliation. Williams and Turner (1974) also demonstrated the utility of Landsat imagery by using spectral signatures of defoliated and nondefoliated areas to produce a map outlining heavy defoliation for a small area in northeastern Pennsylvania.

To examine the feasibility of using Landsat imagery for map defoliation, multispectral scanner (MSS) images were obtained for the area photographed with CIR film. The infrared (IR) and red (R) bands were combined into a ratio mask and density sliced to reflect defoliation levels. A color-encoded scene was generated on a television monitor. This defoliation

map (fig. 3-7) was compared with the map produced from the CIR film (fig. 3-5). The defoliation patterns are quite similar, although the Landsat data were acquired on June 20, 1975, and the film on July 1, 1975—a difference of 11 days.

In addition to MSS images, satellite data were also obtained in digital form on computer compatible tapes (CCT). It appeared that use of the data in this form might produce significant cost savings, because all data manipulation from the SCS technique calibration to map production could be accomplished with a computer; however, before this could be done, it was necessary to develop a mathematical function to relate observed visual estimates of defoliation to reflectance measurements just as was developed for the CIR film. Data from ground plots were used with
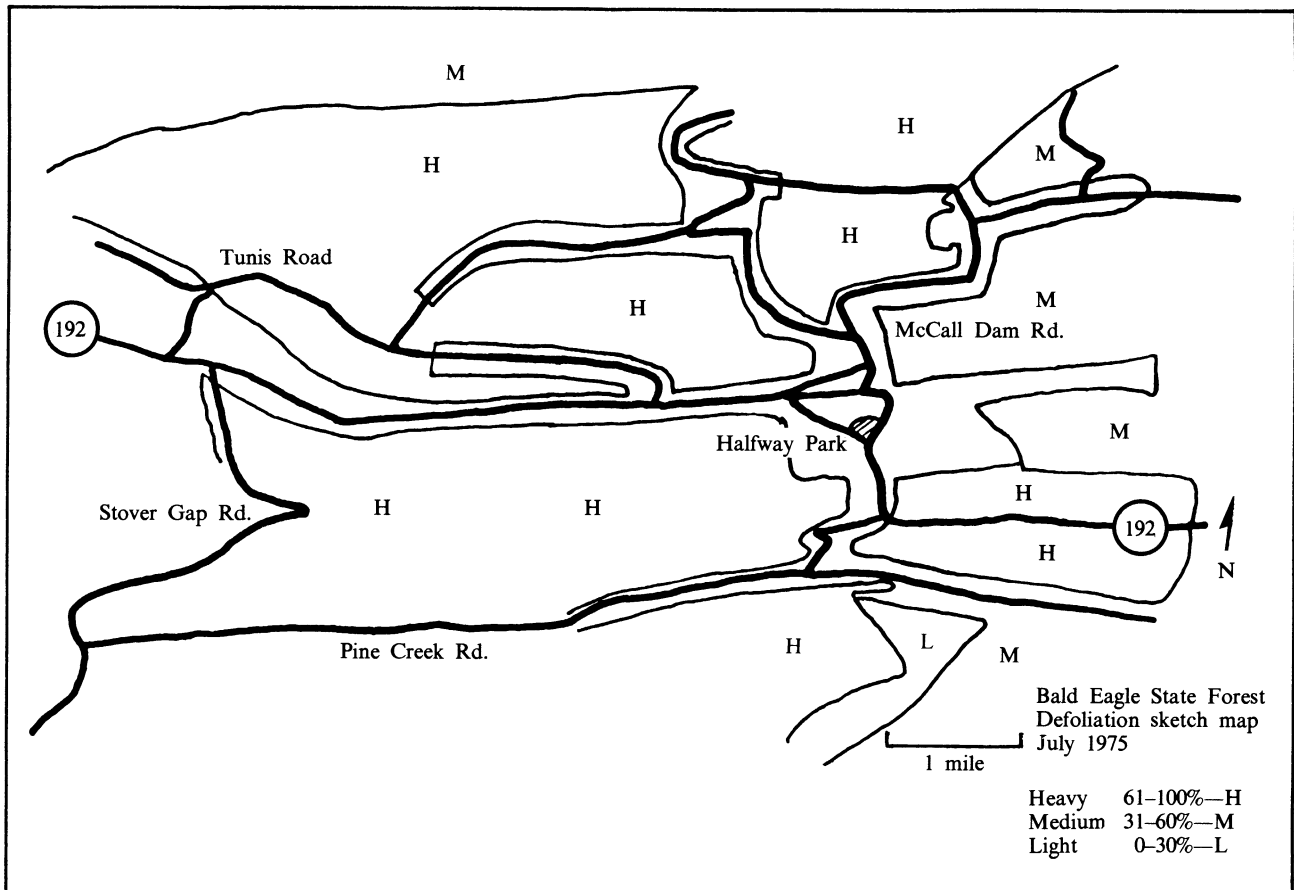
Figure 3-6.—*Aerial sketch map of test site, 1975* .

the June 20, 1975, CCT data. This relationship was found to contain the same reflectance variables as for the CIR film and can be expressed as

percent defoliation $(CCT) = [-291.25 + 16.831X$
$$-0.2385X^2 + 0.0010X^3] \times 100$$

where X equals the defoliation/reflectance parameters of the previous equation.

This third-order polynomial provided a correlation coefficient (R^2) of 0.79 (Walker et al. 1978).

To demonstrate the validity of this measure, a photometric interpretation map was prepared from CCT data for comparison to an aerial sketch map of the same area in central Pennsylvania for 1976 conditions. The aerial sketch map was a composite

from crown conditions observed from flights made on June 4 and 5, 1976, and on July 18, 1976 (fig. 3-8, A). The photometric map used July 19, 1976, CCT data (fig.3-8,B). Entomologists and foresters working in the area agreed that peak defoliation occurred between June 26 and July 2, 1976. The timing of the aerial flights makes comparisons difficult because this map is an ex post facto description of crown conditions tempered by observations of early defoliation and observed refoliation patterns. Even with aerial photographs and photometric interpretation methods, this type of correlation has not been successful (Walker 1976).

However, some inference is possible, although the maps do not permit a valid comparison. The photometric map shows much more area in the
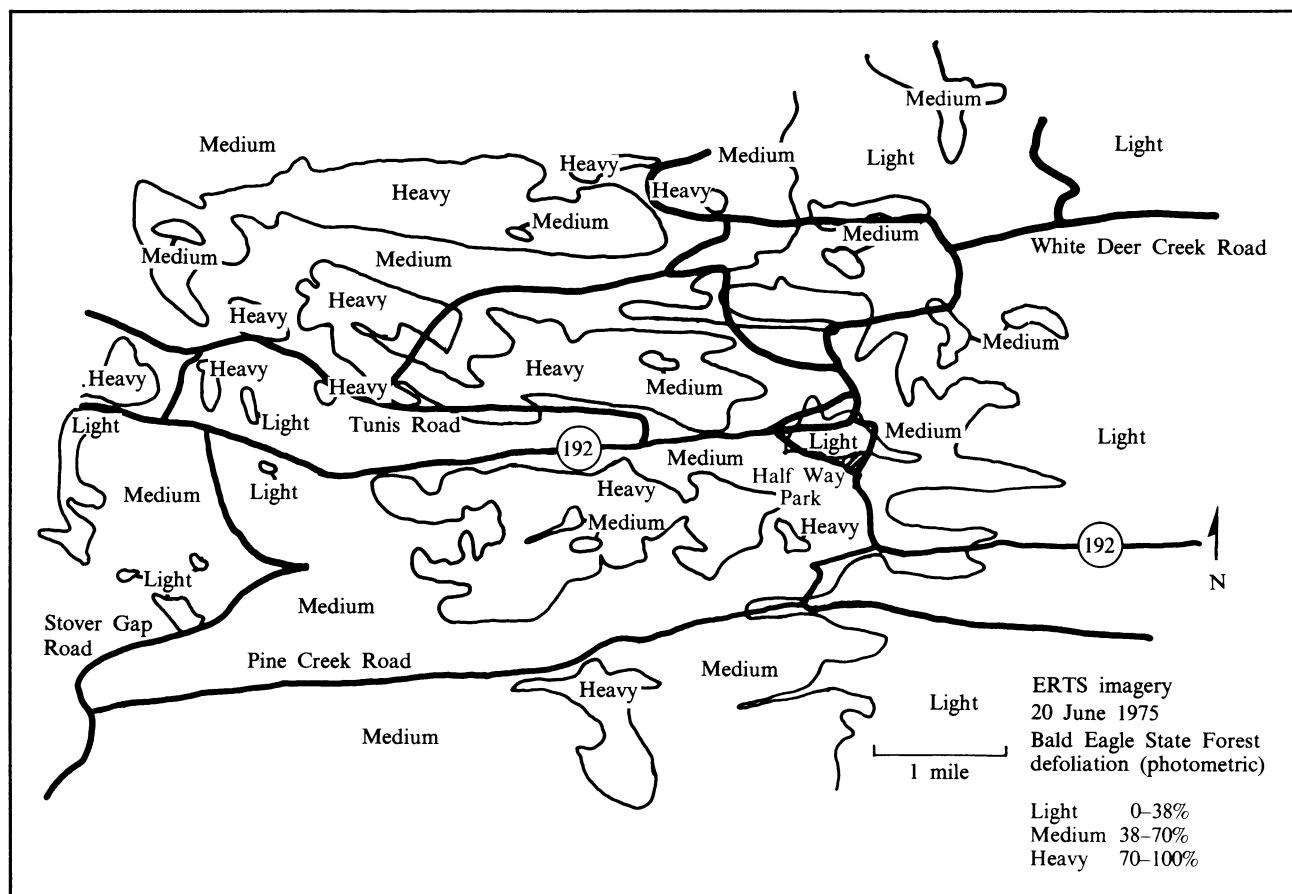
Figure 3-7.—*Photometric defoliation map from June 20, 1975, Landsat imagery of test site.*

heavy-defoliation category. This might reflect the stress condition exhibited by the stands as the result of 1 or 2 years of defoliation. This condition would not be evident in the visual assessment. Many of the light and moderate areas are in close agreement, but the photometric map provides much more detail.

The detail that can be achieved with the CCT data is demonstrated in figure 3-9, which is a portion of figure 3-8, *A*. This raw computer printout has been shaded manually to aid in visualizing the three general defoliation categories—low, medium, and high. Each numeral on the printout represents an approximate 10-percent increment in defoliation. This is the average defoliation level over an approximate 0.5-ha resolution element (pixel) of forest canopy. For this demonstration, every line of data was used, but only

every other pixel was evaluated and printed. The low and medium-to-heavy defoliation patterns match fairly well with the sketch map, but the aerial observer cannot sketch such detail on a map. In the future, investigators might consider using an algorithm to classify broad areas (20- to 40-ha blocks) on a scene into a single defoliation category based on the probability of pixels classified in each category. The resulting map would lack the detail, but patterns would agree more closely with what the aerial observer views and records on the sketch map.

Another area (fig. 3-8, *B*) was mapped with the information content reduced by another factor of two by printing only every other line of data and only three levels of defoliation were encoded (heavy—*x*, medium—*o*, and light—no symbol). In the area of
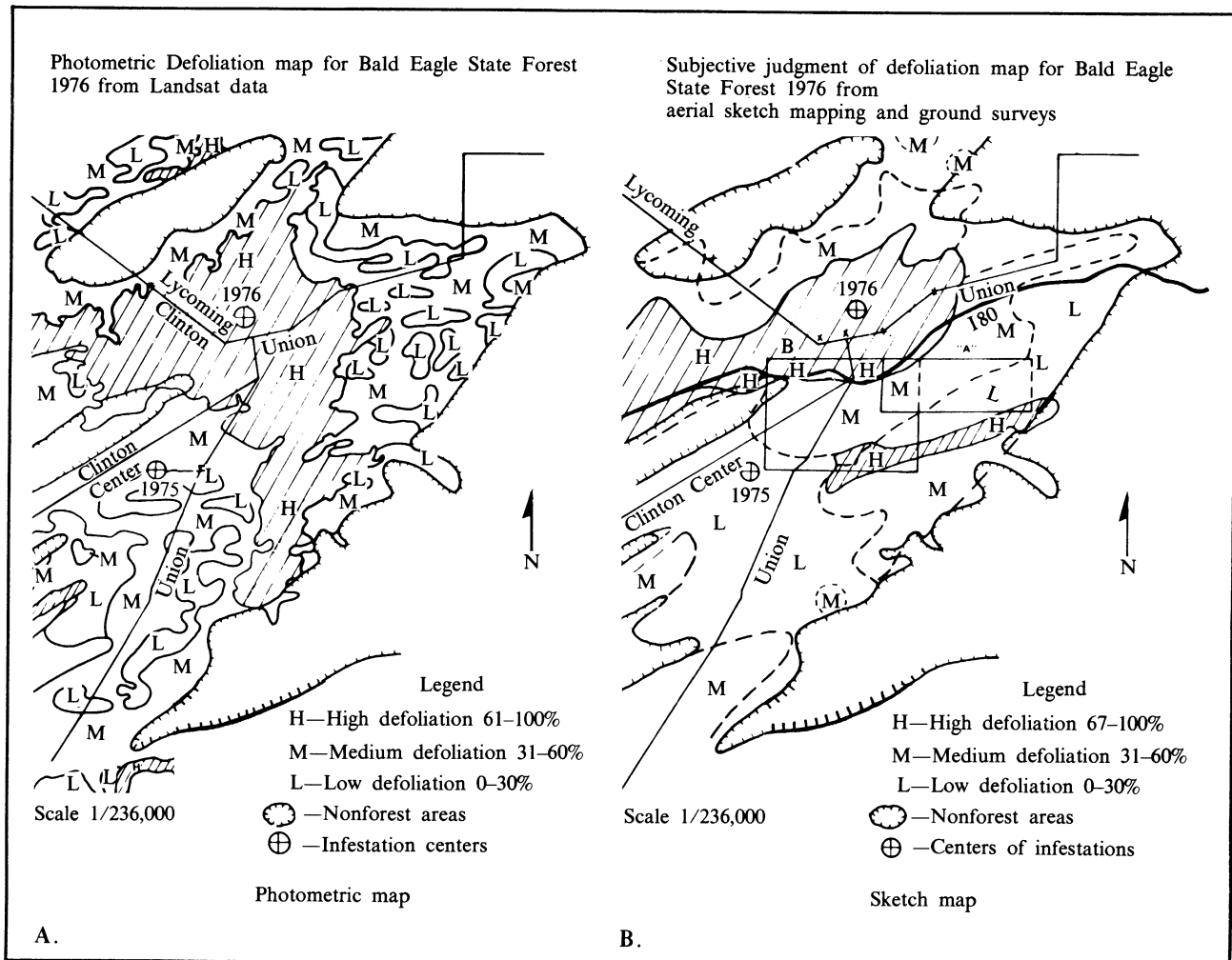
Figure 3-8.—*Comparison of sketch* (A) *and photo-metric* (B) *defoliation maps, 1976.*

overlap with the first printout, the pixels (symbols) shown in any one line of data are the pixels not printed in figure 3–10. Even with the further reduction in data lines printed and the use of pixels among those printed in figure 3–9, the general patterns of heavy and medium defoliation in the area persist.

Recommendations for Implementing System

The necessary cost studies of the various aerial methods for detecting and assessing defoliation have not been made, but some cost figures are available and comparisons can be made if a few assumptions are allowed.

Aerial sketch mapping is the only method employed routinely to locate and rate gypsy moth defoliation. Costs are difficult to find in the literature and must be inferred from the amount spent for the forested area available. Walker et al. (1977) assumed a mean value of $1.22 per 405 ha from information available for the State of Pennsylvania. These costs appear to be reasonable for most States in the Northeast where two observers rent a light aircraft and fly over forested areas to locate defoliation. They record the defoliation by predetermined categories on maps. Once on the ground, they transfer this information to other maps, reconcile differences, and forward this

Legend	Raw data			Encoded defoliation		
Blank less than 10%	6	60–70%				
1	10–20%	7	70–80%	Blank to 2	Low	
2	20–30%	8	80–90%	3 to 5	Medium	
3	30–40%	9	90–100%	6 to 9	High	
4	40–50%	*	Nondefoliation			
5	50–60%					
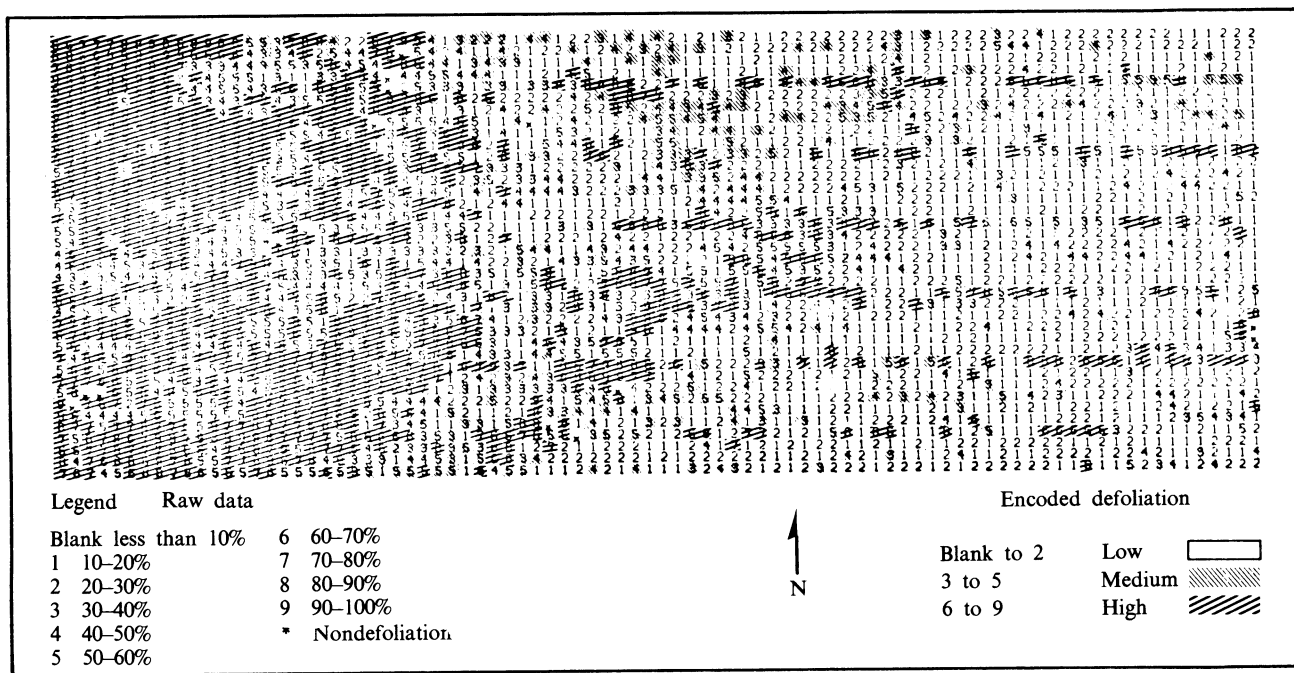
Figure 3-9.—*Digital printout and mapping of gypsy moth defoliation* (*location fig. 3-8*,A).

map to a central office that compiles a picture of defoliation for the State.

The time required to obtain the defoliation data is variable and depends on many factors. Peak defoliation can occur at different times within a region and depends on temperature differences resulting from aspect, topography, and geographic location. Furthermore, weather and visibility problems can extend observation times. As a result, the conditions on an overall map may describe a temporal period of 4 to 5 weeks that represents the "best estimate" of conditions.

To acquire CIR aerial photographs by jet aircraft for a State such as Pennsylvania, which has 6,879,900 ha of commercial forest, would cost about $110,000, or $11 per linear km (Walker et al. 1977). The cost per 405 ha would be $0.65. To this figure must be added the film and processing costs. Weather and cloud conditions would also influence the mission and film acquisition. A considerable number of photographs would have to be interpreted before the defoliation

Legend

xxxx High defoliation (over 60%)
Medium defoliation (30 to 60%)
Low defoliation (less than 30%)
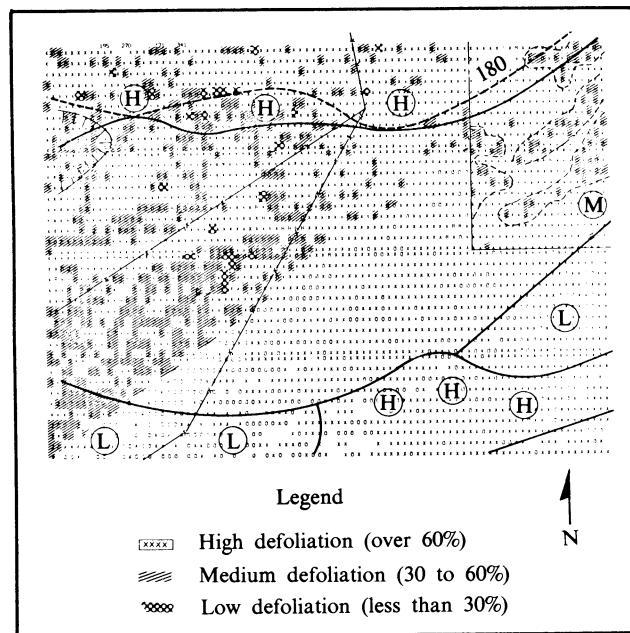
Figure 3-10.—*Digital printout and mapping of gypsy moth defoliation* (*location fig. 3-8,B*).

picture of the State would be available. Once defolia-
tion levels are mapped, areas can easily be measured
with an electronic planimeter. If objective photomet-
ric interpretation is applied to this film, correction
must be made for the illumination look angle problem
present on the aerial photographs. The correction
would add an additional step to the calibration proce-
dure but could be handled by a computer program
with little addition to the overall cost.

Mathematical relationships and demonstrations
have shown that there are quantitative relationships
between forest crown condition and the spectral
reflectance of the forest canopy as measured by pho-
tometric interpretation methods for CIR film, Land-
sat MSS imagery and CCT data (Talerico et al. 1977,
Walker et al. 1978). Visual analysis of MSS imagery is
as subjective as aerial sketch mapping, and the same
categories of defoliation can be distinguished. The
cost of this type of assessment is about $0.36 per 405
ha (Walker et al. 1977).

A more objective analysis of Landsat data is possi-
ble with photometric interpretation methods. If only
the infrared-to-red ratios are used to map two levels of
defoliation—31 to 60 percent and 61 to 100 percent—
costs on the order of $0.50 per 405 ha are possible
(Talerico et al. 1977). Using the full defoliation mea-
sure for the CCT's should not increase this cost. Cost
reductions seem possible through software streamlin-
ing and through the use of only part of the Landsat
digital data. Mapping only these levels would be com-
parable to the results of aerial sketch maps, but this
map would more accurately depict crown conditions.
These estimated costs for gypsy moth defoliation
mapping are tabulated for quick comparisons (table
3-1).

Of course, all methods should be supported by
ground information to verify the cause of defoliation
and the intensity.

Future defoliation assessment systems should
consider some form of Landsat data collection and
employ SCS techniques. Costs are favorable; the
Landsat product is well documented, and availability
should improve. Cloud-cover problems might be
overcome through additional satellite coverage or

Table 3-1.—*Comparison of methods and costs for acquiring
and mapping gypsy moth defoliation data*

Methods of estimating defoliation and area by categories	Estimated cost per 405 ha
Aerial sketch mapping	$1.22
Jet aircraft with CIR film	.65[1]
Landsat	
MSS imagery	.36[2]
CCT digital	.50[3]

[1]Factors for film and processing not included.
[2]Utilizing visual interpretation of imagery.
[3]Cost reduction appears possible.

perhaps by the use of overlap between the preceding
and succeeding daily passes. The overlap might be
sufficient at the latitude of the Northeast to obtain an
impression of how crown conditions are changing
until the next clear scene is available. Another
alternative would be to monitor Landsat data closely
for cloud cover over the area of interest. If the records
show greater than 30 percent cloud cover, sketch
mapping or photo flights might be used to acquire the
data if timing is critical. This would require close user
contact and cooperation between NASA and the user
community. Again, whatever system is employed,
ground checking is necessary to verify defoliation
conditions.

Egg-Mass Density/Defoliation Relation-ships

Robert W. Wilson and Robert L. Talerico

The ability to predict the degree of defoliation from
egg-mass numbers is useful in planning gypsy moth
management activities. The relationship between egg-
mass density obtained by the FVP method and
defoliation the following year is shown in figure 3-11,
which reflects central Pennsylvania conditions. The
coefficient of determination (R^2) for this relationship
is 0.73, a promising relationship considering all fac-
tors—weather, parasites, predators, dispersal, disease,
etc.—acting upon the populations and affecting final
forest defoliation levels.
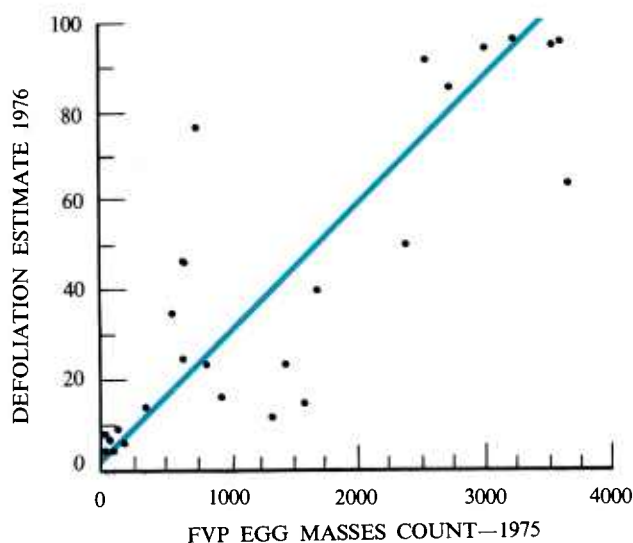
Figure 3–11.—*Relationship between FVP egg-mass counts and subsequent defoliation.*

Management Tools
Robert L. Talerico

Coping with a pest problem has become very complex for the forest or pest manager. The ultimate decision must balance the cost of control against economic, environmental, and social values. Many unknowns are involved because all the interactions that may be involved are just beginning to be understood. Although the gypsy moth has been studied for many years, it is still extremely difficult to predict exactly what a specific population will do in terms of subsequent defoliation and mortality to forested community, the effect the control agent may have on the environment, or the influence of the insect on man and his environment.

Gypsy Moth/ Forest Interaction Model

To gain some insight into the defoliation and tree mortality problem that can result from a gypsy moth infestation, historical data (Campbell and Valentine 1972) has been assembled and analyzed to provide guidelines for decisionmakers to use in assessing current conditions. Tabular data assembled by Campbell and Valentine (1972) describe how tree condition and mortality differ over time with varying levels of defoliation history. These same data were also used to develop an empirical model to simulate the gypsy moth forest interaction in many stands and over many years to yield estimates of future egg-mass density, defoliation, tree condition, and tree mortality (Valentine and Campbell 1975). This model will also yield frequency distributions of expected egg-mass density and defoliation levels for one stand in 1 year.

Other models and procedures have been suggested for use by decisionmakers. Valentine (chapter 3, A Model of Oak Forest Growth Under Gypsy Moth Influence) has proposed tree and insect models that are formulated on biological and physiological processes. Experiments are underway to refine and validate the model parameters so that they truly reflect biological conditions.

Decision Tree Methods

The decision tree technique is a structural method for making a choice among alternatives when many uncertainties exist. For gypsy moth control efforts, the underlying problem is the uncertainty about what level of defoliation will result. With this method, a benefit/cost comparison of the alternatives is possible. The method has been demonstrated on a hypothetical gypsy moth problem using published data (Talerico et al. 1978). The subjective judgments of probabilities used in the decision tree analysis can be replaced by probabilities generated with a process or systems model. Valentine et al. (1977) describe this process and a system model that is compatible with the decision model.

A Model of Oak Forest Growth Under Gypsy Moth Influence
Harry T. Valentine

Introduction

Impact assessment of reductions in forest growth and yield caused by insect defoliation is needed to

facilitate forest management planning. One cannot measure impact, however, because the growth or yield of a forest stand that should be expected in the absence of the insect is never known. One must therefore resort to a grandiose experiment or, better, to a model to predict the expected growth and yield, and then compare these with the actual growth and yield influenced by the defoliating insect.

In order to assess the influence of gypsy moth on oak forest productivity, a model was synthesized that simulates the growth of individual trees in an evenaged oak forest stand. The rates of change of tree components are described by difference and differential equations.

Most extant forest growth models, the purposes of which are the generation of yield tables, consist of systems of empirical equations based more on statistical than biological rationale. The parameters are estimated from data by least squares or maximum likelihood techniques. With these empirical approaches, there is more concern for precise predictions of yield than for description or understanding of the biological processes that produce the yield.

This forest growth model was developed using a mechanistic approach (although it is not without empirical compromises). Rather than fit curves to data, an attempt was made to quantitatively describe biological processes. The function that describes the growth of trees is based on physiological processes, and the influence of gypsy moth on that growth is described by functions that correspond to real processes—for example, larval browsing, larval growth, and larval mortality. Parameters of mechanistic functions are sometimes measured and sometimes precisely estimated following experimentation or sampling. Initial parameter values are often subjectively assigned and then adjusted during sensitivity analyses.

This model is designed to describe gypsy moth influence in existing oak forests. It starts with a description of an existing stand that is sampled as fixed-area plots. It requires two measurements of the diameter at breast height (d.b.h.) of each tree separated by 5 years or more. The two measurements are used to calibrate the diameter-squared growth rate (the difference between the tree's diameter squared at two different times) of each tree. Tree growth is influenced by crowding, which is estimated with a simple stocking formula requiring no intertree distances. (Stocking is the ratio of current tree density to the assumed density for optimal stand growth.) Consequently, the sampled plots should be 0.1 ha or larger so that stocking will be computed accurately and so that the death of a few trees and the concomitant reduction in crowding will not unduly influence the simulated growth of the residual stand.

Assessment of gypsy moth impact is accomplished by running the forest growth model twice—with and without gypsy moth influence—using the same initial conditions each time. The change in forest growth due to gypsy moth influence is obtained by comparing the two runs or, preferably, many pairs of runs. Control decision rules are evaluated by simulating forest growth a third time from a given set of initial conditions. The influence of gypsy moth on forest growth and the influence of control treatments on the gypsy moth population are simulated. A control treatment is applied when the conditions of the decision rule are met. The cost effectiveness of the control decision rule can be evaluated by comparing the rates of return on forest management investments with and without control treatments.

Forest growth (which is the combined growth of individual trees) is simulated in the time step of 1 year. If gypsy moth browsing is predicted for the growing season, this process is modeled with a system of differential equations. The solutions of the differential equations are used to adjust the growth of the individual trees of the stand. The amount of adjustment depends on the amount of foliage consumed by gypsy moths. How this is done will become apparent after a discussion of the forest growth model and the differential equation model, hereafter called the gypsy moth submodel.

The Forest Growth Model

The fundamental equation of this model has the yearly increase in a tree's volume ($\triangle V$) equal to the total net photosynthate (P) exported from the foliage,

less respiration (R). Here respiration is defined as the use of P for purposes other than wood production, such as foliage production, maintenance respiration, and net change in stored substrate, so that

$$(1) \quad \triangle V = P - R.$$

Both P and R are assumed to be measurable in units equivalent to a dimensional measure of wood volume. Moreover, a tree is assumed to maintain a level of stored substrate equal to αV. Rewriting (1) with R expressed as a quantity of substrate equivalent to wood volume yields

$$(2) \quad \triangle V = P - (\lambda + \eta + \rho + \mu)V$$

or, letting $a_2 = \lambda + \rho + \mu$,

$$\triangle V = P - a_2 V - \eta V.$$

$\lambda, \eta, \rho,$ and μ are parameters, and V is tree volume, so that

λV = Substrate used in first flush or leaf production.

ηV = Substrate used in second flush or leaf production (refoliation), if any.

ρV = Substrate used in maintenance respiration of all living tissue other than foliage.

μV = Net increase in stored substrate that maintains the level of stored substrate optimally at αV.

A modification of (2) is necessary to describe tree growth when defoliation or other processes cause respiration to exceed photosynthate production:

$$P - a_2 V - \eta V < 0.$$

In that case, growth is zero and stored substrate is used to maintain living tissue:

$$\triangle V = 0 = P - a_2 V - \eta V + \alpha' V$$

so that

$$\alpha' V = a_2 V + \eta V - P$$

where $\alpha' V$ is a deficit in the optimal quantity of stored substrate. In the following year, (2) is modified so that

$$(3) \quad \triangle V = P - (a_2 + \eta + \alpha')V$$

or, if the tree is defoliated again, so that

$$P - (a_2 + \eta + \alpha')V < 0$$

then

$$\triangle V = 0 = P - (a_2 + \eta + \alpha')V + \alpha'' V$$

where $\alpha'' V$ is the new deficit in the optimal quantity of stored substrate. If at any time stored substrate is totally depleted, the tree is presumed dead and treated as such.

Diameter-Squared Growth Rate

To put (2) in terms of measurable tree dimensions, it was assumed that P, in the absence of defoliation, should be proportional to the foliage quantity of the tree, which in turn is proportional to d.b.h. squared (D^2) (Shinozaki et al. 1964). It was also assumed that V equaled $D^2 H$, where H is tree height, yielding

$$(4) \quad \triangle V = a_1 D^2 - a_2 D^2 H.$$

If V equals $D^2 H$, then

$$\triangle V = \triangle D^2 H + \triangle H D^2 + \triangle D^2 \triangle H.$$

Assuming height growth precedes D^2 growth, the contribution of D^2 growth to volume increase is $\triangle D^2 H$. Substituting $\triangle D^2 H$ for $\triangle V$ in (4) and solving for $\triangle D^2$ yields

$$(5) \quad \triangle D^2 = a_1 D^2 / H - a_2 D^2.$$

According to (5), D^2 (basal area of a tree) increases from year to year until a_1 / H equals a_2. Therefore, when maximum height is reached, which in reality

would mean no apical growth and consequently no foliage production, all volume growth of a tree should cease.

If the model used (5) to estimate $\triangle D^2$, a height estimator would be needed. For the present purpose of the model, H was replaced in (5) by $D^{2/3}$, making use of Greenhill's (1881) height/diameter relation, yielding

$$(6) \quad \triangle D^2 = a_1 D^{4/3} - a_2 D^2.$$

The quantity of photosynthate produced by a tree over the course of a growing season will depend, to a large extent, on the amount of light energy it receives. Accordingly, potential tree growth should vary over time as the stocking level of the stand changes. To account for the effects of stocking, a scaling factor, C, was added to (6) to give a simulated response to changes in stand stocking:

$$(7) \quad \triangle D^2 = a_1 C D^{4/3} - a_2 D^2.$$

C varies between 0 and 1, and is described by the following equation:

$$(8) \quad C = b_1/(1 + (S/b_2)^{b_3}) + b_4/(1 + (S/b_5)^{b_6})$$

where b_1, b_2, ... b_6 are parameters with the constraint that $b_1 + b_4 = 1$. S is the stocking percent of the stand, which is calculated with Gingrich's (1967) stocking formula for upland oak stands.

Calibration

Because site and inherent production efficiencies should be reflected in tree growth rates, a procedure was developed to calibrate individual tree D^2 growth rates from two measurements of d.b.h. separated by 5 years or more, obviating the need for specific site information. In the D^2 growth equation (7), the respiration parameter (a_2) is assumed to be constant for each species. The photosynthesis parameter (a_1) is calculated for each tree with an iterative procedure.

First, each tree's growth is projected from year 0, the year of the first measurement, until the year of the remeasurement, using "average tree" parameter values. Next the ratio of predicted to actual D^2 growth (r) is computed, and then a_1 is adjusted using the predicted D^2 growth ($\triangle D^2$) from year 0 to year 1 as

$$(9) \quad a'_1 = (\triangle D^2 r + a_2 D^2)/D^{4/3} C$$

where D is the tree's d.b.h. in year 0.

The whole process is repeated until the predicted d.b.h. of each tree is very close to the second observed value.

Individual tree merchantable volume is not a real component of the model but is estimated for impact assessment purposes in terms of cubic feet. Because tree height is ignored in this version of the model, tree volume is computed from a function developed by Meyer and Kienholz (1944) for the generation of local volume tables for Connecticut. This function requires only diameter as an independent variable. The parameters of the function vary among species.

Tree Mortality

The forest growth model has provisions for the removal of trees. Reductions in the number of trees can occur in three ways. Thinnings can be simulated to reduce the number of trees. This not only increases the growth of the remaining trees (provided they are not spread too thin to begin with) but also reduces their chances for natural mortality, which occurs during simulation if a tree's stored substrate becomes totally depleted. The third way tree numbers are reduced is through a procedure that randomly kills trees, each with a yearly probability of $k_1 + k_2 D_p$, such that $k_1 + k_2 D_p \ll 1$, where D_p is the percent defoliation of the tree, and k_1 and k_2 are small constants. This random mortality is imposed to account for mortality caused by secondary pests such as the twolined chestnut borer (*Agrilus bilineatus*) and shoestring fungus (*Armillaria mellea*), which attack defoliated oak trees more frequently than nondefoliated trees (Nichols 1968, Dunbar and Stevens 1975).

The Gypsy Moth Submodel

The gypsy moth submodel consists of a set of differential equations and certain ancillary functions that provide either initial values of the components (whose changing values are described by the differential equations) or values of parameters. It is an extension of a model reported previously (Valentine et al. 1977). The differential equations are used to describe the defoliation process and the resulting reduction in photosynthate production during the growing season. Typically, over time a numerically changing population of larvae consumes expanding foliage at an increasing rate per larva until all foliage is consumed, or until all larvae die or pupate. The growing season is currently measured in days (t). (However, this will be changed to degree-days.)

The defoliation process is modeled individually for each species of a plot. A proportion of the gypsy moth population (assumed hatched from viable eggs produced the year before) is allocated to each species at the start of the integration interval (the growing season). Each species allocation is based on its proportion of the total basal area of the plot and relative defoliation ratios (Campbell and Sloan 1977). This procedure is used in lieu of complex models of the gypsy moth dispersal process.

Submodel Components

The components of the gypsy moth submodel are:

$H(t)$= Number of healthy gypsy moth larvae per hectare.

$V(t)$= Number of virus-infected gypsy moth larvae per hectare.

$C_1(t)$= Accumulative consumption by a single gypsy moth larva, expressed in kilograms per hectare.

$C_N(t)$= Accumulative consumption by the gypsy moth population, expressed in kilograms per hectare.

$W(t)$= Average dry weight of a gypsy moth larva, expressed in grams.

$F(t)$= Dry weight of foliage that would exist in the absence of insect consumption, expressed in kilograms per hectare.

$F^*(t)$= Dry weight of actual foliage, expressed in kilograms per hectare.

$F_v(t)$= Dry weight of actual foliage contaminated with gypsy moth nucleopolyhedrosis virus, expressed in kilograms per hectare.

$F_1(t)$= Dry weight of actual foliage contaminated with insecticide, expressed in kilograms per hectare.

$F_2(t)$= Dry weight of second-flush foliage (refoliation), expressed in kilograms per hectare.

$Q(t)$= Index of foliage nutritional quality, which takes values between 0 and 1.

$P(t)$= Photosynthate produced by $F(t)$.

$P^*(t)$= Photosynthate produced by $F^*(t)$.

$F_s(t)$= Amount of $F(t)$ that is senescent and nonproductive.

The Defoliation Process

Given $H(t)$ and $V(t)$ for any value of t, then the foliage consumption by the moth population and the growth of unconsumed foliage of the trees are described by the following set of differential equations:

$$(10) \quad dC_1/dt = g_1 WF^*/(g_2(H + V)W + F^*) \quad t < g_3$$

$$dC_1/dt = 0 \qquad\qquad t \geq g_3$$

$$(11) \quad dC_N/dt = (H + V)dC_1/dt$$

$$(12) \quad dW/dt = g_4 dC_1/dt(Q/g_5 + Q) - g_6 W$$

$$(13) \quad dF/dt = g_7 F^{g_8} - g_9 F$$

$$(14) \quad dF^*/dt = (F^*/F)dF/dt - dC_N/dt + dF_2/dt$$

$$(15) \quad dF_2/dt = 0 \qquad\qquad F^*/F > g_{10}$$

$$dF_2/dt = 0 \qquad\qquad F^*/F = g_{10}; t \leq A$$

$$dF_2/dt = g_{12} F_2^{g_{13}} - g_{14} F_2$$
$$\qquad\qquad F^*/F < g_{10}; t > A + g_{11}$$

$$(16) \quad dQ/dt = -g_{15} dF/dt.$$

Change in accumulative consumption per larva (10) is proportional to average larval dry weight ($g_1 W$) modified by $F^*/[g_2(H+V)W+F^*]$. As defoliation becomes more complete, this latter expression causes the consumption per larva to be slowed, as it was assumed that a larva should have difficulty finding enough to eat when the demands for food by the population approach or exceed the food available. When all food is depleted, consumption ceases—that is, if $F^*=0$, then $dC_1/dt=0$. When $t=g_3$, all larvae are assumed to pupate, so consumption ceases. Change in the accumulative consumption by the population is described by (11). It is simply the number of larvae per hectare multiplied by dC_1/dt.

The change in dry weight of a larva (12) is proportional to the amount of food it eats, modified by a function of nutrition (Q/g_5+Q) less respiration ($g_6 W$). When food is depleted or scarce so that

$$g_4 dC_1/dt(Q/g_5 + Q) < g_6 W,$$

the larva will lose weight. If the nutrition of the food becomes low, assimilation is slowed and could also result in larval weight loss.

Foliage growth that would be expected in the absence of gypsy moth consumption is described by the Bertalanffy growth-rate equation (13). This is merely a convenient way to describe expected foliage growth, which does not depend on other components of the model. Changes in the index of foliage nutrition quality (16) occur as the foliage grows and, therefore, reflect the decreasing dry-weight density of many minerals and amino acids found in expanding tree foliage. Consequently, small young leaves are considered more nutritious than large mature leaves.

Growth of actual foliage, partially consumed or not, is described by (14). If accumulative consumption (C_N) equals 0, then actual foliage (F^*) equals expected foliage (F). If $C_N>0$, so that $F^*<F$, then the growth of $F^*=dF/dt$, reduced by the factor F^*/F,—that is, the unconsumed fraction of foliage grows at the same rate that it would if the consumed fraction remained. While growth is occurring, F^* may be reduced by additional consumption. If foliage growth has stopped so that $dF/dt=0$, then dF^*/dt equals the

change in accumulative consumption: $-dC_N/dt$. Refoliation (dF_2/dt) in gypsy moth infestations usually occurs after consumption ceases. Refoliation (15) is assumed to start when $t=A+g_{11}$, after F^*/F decreases to a threshold (g_{10}) at $t=A$.

Larval Population Changes

Changes in H, V, and F_v are described by the following differential equations when control treatments are not applied (Etter 1977):

(17) $dH/dt = -g_{16}H - (HF_v/F^*)(dC_1/dt/g_1 W)$
$\qquad - g_{17}H(1 - dC_1/dt/g_1 W) \qquad t < g_3$

$dH/dt = 0 \qquad t \geqslant g_3$

(18) $dV/dt = -g_{16}V - g_{18}V + (HF_v/F^*)$
$\qquad (dC_1/dt/g_1 W)$
$\qquad - g_{17}V(1 - dC_1/dt/g_1 W) \qquad t < g_3$

$dV/dt = 0 \qquad t \geqslant g_3$

(19) $dF_v/dt = g_{18}VW(1 - F_v/F^*) - g_{19}F_v$
$\qquad - dC_N/dt(F_v/F^*) \qquad t < g_3$

$dF_v/dt = -g_{19}F_v \qquad t \geqslant g_3$

With the exception of the transition of larvae from the healthy component in (17) to the virus-infected component in (18), all changes in H and V result in a net decrease in the population. Barring net immigration of larvae into the plot resulting from their dispersal process, this is as it should be. Reduction in larval numbers is due to density-dependent mortality ($-g_{16}H$ and $-g_{16}V$), virus-caused mortality ($-g_{18}V$), and starvation [$-g_{17}H(1-dC_1/dt/g_1 W)$]. Starvation is nil if $dC_1/dt=g_1 W$, and maximal when $dC_1/dt=0$. The density-dependent mortality is assumed to account for all mortality due to processes other than virus-caused mortality or starvation.

The transition of larvae from the healthy component to the virus-infected component depends on the density of healthy larvae (H), the ratio of virus contaminated to total foliage (F_v/F^*), and the consumption rate per larva ($dC_1/dt/g_1 W$). Larvae are assumed to ingest the contaminated foliage, which

results in disease and eventually death. When $dC_1/dt = g_1 W$, there are no constraints on food availability. Consumption is maximal and, therefore, infection is maximal. When $dC_1/dt = 0$, there is no increase in infected larvae.

Change in the quantity of virus-contaminated foliage (19) is due to new contamination by dying larvae, natural decontamination, and consumption. New contamination $(g_{18} VW(1 - F_v/F^*))$ is proportional to the dry weight of larvae dying from virus multiplied by the ratio of uncontaminated to total foliage. The rate of decontamination is assumed proportional to the amount of contaminated foliage $(-g_{19}F_v)$ and consumed foliage is assumed to contain the fractional amount of contaminated foliage (F_v/F^*).

If control treatment effects are modeled, the differential equations are integrated to the time of treatment: $t = t_c$. From t_c until the time of pupation, changes in H, V, and F_1 are described by:

(17a) $dH/dt = -g_{16}H - (HF_v/F^* + g_{20}HF_1/F^*)$
$\qquad (dC_1/dt/g_1 W)$
$\qquad - g_{17}H(1 - dC_1/dt/g_1 W)$.

(18a) $dV/dt = -g_{16}V - g_{18}V + (HF_v/F^*$
$\qquad - g_{20}VF_1/F^*)(dC_1/dt/g_1 W)$
$\qquad - g_{17}V(1 - dC_1/dt/g_1 W)$.

(20) $dF_1/dt = -g_{21}F_1 - dC_N/dt(F_1/F^*)$.

Equations (17a) and (18a) differ from (17) and (18) by the additional terms for mortality resulting from consumption of pesticide: $(-g_{20}HF_1/F^*)$ and $(-g_{20}VF_1/F^*)$, respectively. Pesticide-induced mortality depends on the larval population $(H + V)$ and the ratio of pesticide-contaminated foliage to total foliage (F_1/F^*). The quantity of pesticide-contaminated foliage is assumed to decrease because of chemical breakdown and cleansing $(-g_{21}F_1)$ and consumption: $dC_N/dt(F_1/F^*)$. If a control treatment consists of virus application, (20) is not needed. Instead, the quantity of virus-contaminated foliage (F_v) is increased, and the orginal equations are used to describe changes in the system's components after the treatment.

Photosynthate Production

The solutions of the differential equations that describe expected and actual photosynthate production and foliage senescence are used to compute the ratio: $P^*(t_2)/P(t_2)$, where t_2 is the end of the growing season. Because only a ratio is needed to adjust tree growth, which is the primary purpose of the gypsy moth submodel, scaling parameters have been omitted in the equations describing changes in P and P^*. Thus

(21) $\quad dF_s/dt = 0 \qquad\qquad\qquad t \leqslant g_{23}$

$\qquad dF_s/dt = g_{22}F_s(1 - F_s/F) \qquad t > g_{23}$

(22) $\quad dP/dt = F - F_s$

(23) $\quad dP^*/dt = (C + 1)(F^*/F)/(C + F^*/F)\, dP/dt$

where C = the constraint of stocking on photosynthate production, which is computed with (8).

If defoliation is nil, so that $F^* = F$, then expected and actual photosynthate productions are equal—that is, $dP^*/dt = dP/dt$. If all foliage is consumed, so that $F^* = 0$, then photosynthate production is nil $(dP^*/dt = 0)$. Partial defoliation is assumed to cause an increase in the production efficiency of residual foliage, which would normally be in the lower crowns of trees, as light coming to that foliage should increase. When foliage becomes senescent, so that $F_s = F$, photosynthate production ceases.

After the differential equations are solved numerically, the ratio $P^*(t_2)/P(t_2)$ for each species is multiplied by the photosynthesis expression of the diameter-squared growth rate equation when it is used to increment D^2 of the trees of that species. Thus, when gypsy moth influence is modeled, (7) becomes:

(24) $\quad \Delta D^2 = a_1 CD^{4/3}(P^*/P) - a_2 D^2 - \eta D^2$.

Of course, ηD^2 (stored substrate used in refoliation) = 0, if $F_2 = 0$.

The gypsy moth submodel can stand alone. One only needs to assign values to the differential

equations at time $t=0$ and to indicate how much foliage should exist per hectare after it matures to make the model work.

Solutions of some of the differential equations with and without control treatments imposed were obtained numerically. In all cases, the initial populations were 1,200,000 healthy larvae and 12,000 virus-infected larvae. It was indicated that the dry weight of foliage should reach 2,000 kg per hectare if and when it matured. These solutions are graphed in figures 3–12 through 3–16.

Sensitivity Analysis

This kind of analysis is used to determine how the solutions of sets of equations are affected when the parameter values and the initial conditions are altered one at a time. The solutions are said to be most sensitive to the parameters and initial conditions whose changed values produce the largest changes in the solutions. Accordingly, these are the ones that should be measured or estimated most precisely.

Figure 3-13.—*Solutions of (22) and (23), or* P(t) *and* P*(t), *which are expected and actual accumulative photosynthate production, respectively. Gypsy moth foliage consumption shown in figure 3–12 caused a reduction in the photosynthate production of about 30 percent.*

Figure 3-12.—*Solutions of (11), (13), and (14), or* $C_N(t)$, F(t), *and* F*(t), *with no control treatment imposed. The consumption by larvae was sufficient to cause refoliation, which is why* F*(t), *actual foliage per hectare, increases after accumulative consumption of the larval population* [$C_N(t)$] *ceases to increase.* F(t) *is the amount of foliage that would exist in the absence of gypsy moth.*
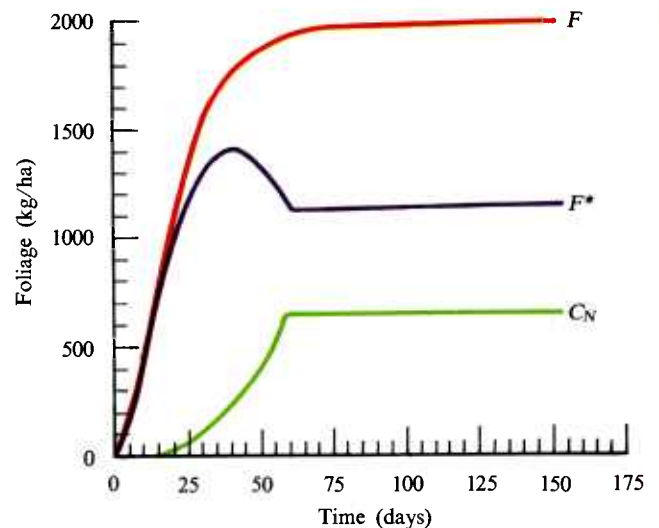
Figure 3-14.—*Solutions of (11), (13), and (14), with a virus control treatment imposed on day 30. The treatment slowed consumption enough to preclude refoliation. The same initial larval population was used in this example as in the example shown in figure 3–12.*

Forest Growth Model

The sensitivity of the forest growth model and the gypsy moth submodel were examined separately. Data were obtained from oak forest stands so that the sensitivity and accuracy of equations (7) and (8) of the forest growth model could be demonstrated simultaneously. The parameter a_2, the exponent of (7), and all the parameters of (8) were varied one at a time in separate runs 5 percent above and below fixed values that, for this analysis, did not vary among species. Two d.b.h. measurements, taken 8 years apart, were used to estimate the parameter a_1 of (7) for each tree after the other parameters were varied. Trees were removed in the year corresponding to when they actually died or where cut as indicated by the data, so that the provisions for random tree mortality would not unduly influence the results. The basal areas of all trees on 20 0.1-ha plots were projected with the model for a period of 16 years. The plot basal area was computed as the sum of the basal areas of all the individual trees. The actual and predicted plot basal areas in year 16 are arranged in table 3-2.

The sensitivity of the basal area projections to changes in the parameters varied from plot to plot. Certain parameter changes affected the basal area projections of plots with high stocking but not those with low stocking. For other parameters, the converse was true. The three parameters that most affected the average basal area projection of all 20 plots were the exponent of (7) and b_2 and b_5 of (8) (table 3-2).

The projection that came closest to the actual basal area occurred when b_5 of (8) was increased by 5 percent. This projection also had the smallest standard error, which means that, on average, the individual plot basal area projections came closest to the actual values after 16 years. When b_5 was decreased by 5 percent, the resulting standard error was the largest of all the projections. This parameter causes tree growth to become slowed when a stand becomes very dense. When its value is increased, the predicted growth of the individual trees will also increase in dense stands.
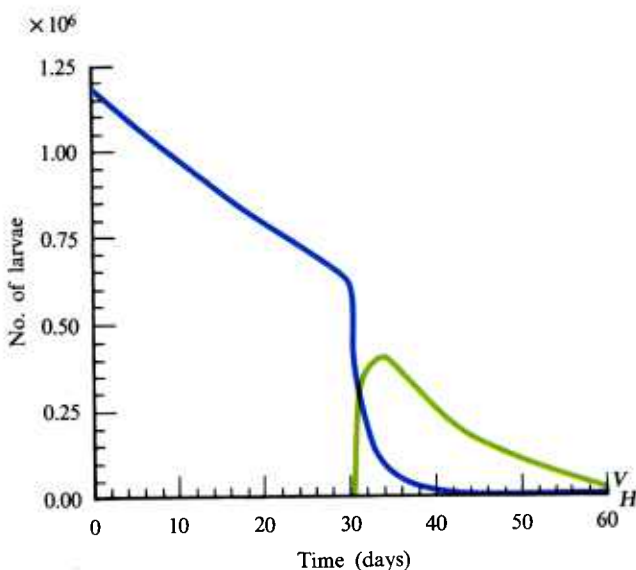
Figure 3-15.—*Solutions of (17) and (18), or* H(t), *the healthy larval population, and* V(t), *the virus-infected population. After a virus control treatment was imposed on day 30, there was a rapid transition of larvae from the healthy to the virus-infected condition and soon afterwards an overall increase in mortality rate.*
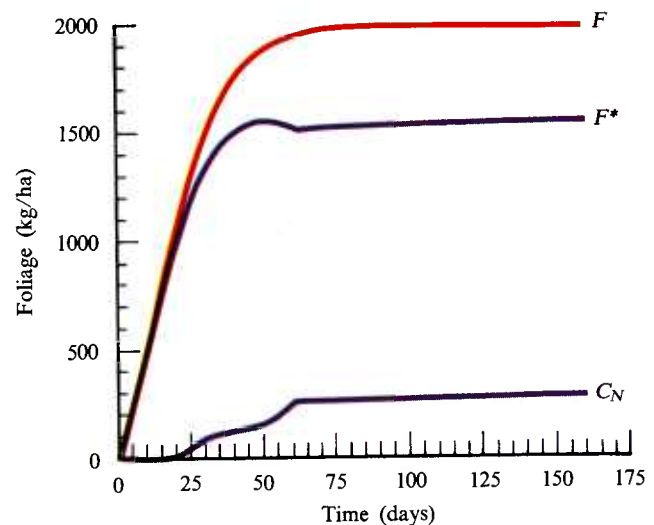
Figure 3-16.—*Solutions of (11), (13), and (14) with a chemical control treatment imposed on day 30. The resulting defoliation level, 1 -* F*(t)/ F(t), *was about 0.25. The same initial larval population was used in this example as in the examples shown in figures 3-12 and 3-14.*

Table 3-2.—*Projections of the basal area of 20 plots*

Plot	BA(0)[2]	Actual BA(16)[3]	Predicted basal area BA(16)[1]						
			Fixed parameters	$0.95(4/3)$[4]	$1.05(4/3)$	$0.95b_2$	$1.05b_2$	$0.95b_5$	$1.05b_5$
1	78.8	111.8	100.8	100.2	101.3	100.8	100.7	99.1	102.8
2	69.2	98.0	97.8	96.6	99.0	98.0	97.6	96.3	98.9
3	59.1	79.0	82.3	81.1	83.4	83.0	81.4	82.0	82.5
4	46.7	75.9	70.3	69.5	71.2	72.8	68.1	70.3	70.4
5	83.9	104.4	104.3	103.7	104.8	104.3	104.2	103.1	105.5
6	77.7	99.6	97.3	96.6	97.9	97.3	97.2	96.0	98.6
7	88.6	93.4	97.5	96.8	98.4	97.8	97.2	96.6	98.2
8	57.8	64.7	63.9	63.2	64.8	64.8	63.2	63.9	64.0
9	56.1	83.3	84.1	82.6	85.7	86.4	81.7	83.8	84.2
10	82.9	106.8	97.5	97.1	98.0	97.5	97.5	95.0	99.3
11	83.4	120.3	115.8	115.4	116.3	115.9	115.8	112.6	119.4
12	66.4	84.2	87.6	86.8	88.4	87.8	87.3	87.2	87.9
13	60.2	80.4	82.1	81.0	83.4	83.2	80.7	81.8	82.3
14	46.6	52.3	46.7	46.1	47.3	46.6	46.6	46.7	46.7
15	85.6	120.0	110.9	110.2	111.6	110.9	110.9	107.8	114.6
16	84.6	113.2	118.0	117.4	119.7	118.1	118.0	115.5	120.8
17	72.4	105.4	110.0	109.1	110.9	110.2	119.8	107.3	112.2
18	59.5	97.8	101.3	100.2	102.5	102.6	99.7	100.3	102.0
19	51.8	91.2	92.8	91.5	94.0	96.3	89.4	92.4	93.0
20	89.2	105.5	100.0	99.4	100.5	100.0	100.0	98.6	101.9
Mean	69.0	94.4	93.0	92.2	94.0	93.7	92.4	91.8	94.3
Standard error			5.06	5.20	5.15	5.24	5.13	5.85	4.60

[1]Predicted basal areas are arranged according to which parameter value was varied and by how much.
[2]BA(0) is the initial basal area in year (0).
[3]BA(16) is the actual basal area in year (16).
[4]$(4/3)$ is the exponent of equation (7), and b_2 and b_5 are parameters of equation (8). $0.95(4/3)$ means, for example, that this exponent was reduced 5 percent before the projections were made.

An increase in the exponent of (7) decreased the difference between the actual and projected average plot basal areas after 16 years, but the standard error was increased. An increase in b_2 of (8) also decreased the difference between the actual and projected average plot basal areas, but at the expense of an increased standard error. An increase in the exponent of (7) increased the projected basal areas of all the plots, but the increase in b_2 only increased the projected basal areas of the plots with low stocking.

Gypsy Moth Submodel

The primary purpose of the gypsy moth submodel is to estimate the ratio P^*/P for each tree species. Therefore, the sensitivity of this ratio to changes in the

parameter values and in the initial values of the differential equations was examined. The differential equations were solved numerically (by the Runge-Kutta method) after a parameter or initial value was varied either 5 percent above or below its estimated or assigned value. A complete set of solutions was obtained when all the parameters and initial values had been varied both upward and downward.

Ten complete sets of solutions were obtained. For each set, a different initial population of gypsy moths was used, but the same maximum amount of foliage (2,000 kg per hectare) was used in each case. The parameter values of (13), which describes expected foliage growth, were estimated from red oak leaf growth data. For the 10 sets of solutions, the initial healthy gypsy moth populations ranged from 200,000

to 2,000,000 larvae per hectare in constant increments of 200,000. The initial virus-infected gypsy moth populations equalled 1 percent of the initial healthy population in each case.

The ratio P^*/P was found to be most sensitive to changes in the 10 parameters and two initial values listed in table 3-3. The parameters that regulate larval consumption and growth and foliage growth were found to produce the greatest changes in P^*/P. In some cases altered parameter values produced irregular results. For example, decreases in g_1 and g_4, given certain initial gypsy moth populations, produced positive changes in P^*/P. But at other initial populations, identical decreases in g_1 and g_4 produced negative changes in P^*/P.

The irregularities of the responses of P^*/P to some parameter changes were due to refoliation. Refoliation occurs only if defoliation exceeds the threshold

g_{10}. An initial population of about 1,000,000 larvae per ha was sufficient to cause refoliation when none of the parameters or initial values was varied from estimated or assigned values. However, when g_1 and g_4, for example, were varied upward and the initial larval population was about 800,000 (600,000 in the case of g_4), consumption increased enough to cause refoliation, which in turn increased P^*. When these parameters were varied downward and the initial larval population was either about 1,000,000 or 1,200,000, consumption was decreased enough to preclude refoliation.

In a separate analysis, the sensitivity of P^*/P to changes in g_{20} and g_{21}, when insecticide treatments were applied to the foliage 20, 25, or 30 days after egg hatch, was examined. The parameter g_{20} is the intrinsic mortality rate per larva that has consumed foliage contaminated with insecticide. The parameter

Table 3-3.—*Changes in the ratio* P*/ P *resulting from 5-percent increases* (+) *and 5-percent decreases* (-) *in the parameters and initial values of the gypsy moth submodel, arranged according to the initial healthy larval population*

Parameter or initial value		Initial healthy gypsy moth population $\times 10^{-5}$									
		2	4	6	8	10	12	14	16	18	20
g_1	+	-0.05	-0.10	0.00	0.03	-0.08	-0.07	-0.05	-0.04	-0.03	-0.03
	-	.03	.06	.10	.13	.00	-.02	.08	.08	.07	.06
g_4	+	-.04	-.09	.00	.04	-.07	-.06	-.05	-.03	-.03	-.02
	-	.03	.06	.09	.11	-.02	-.04	.07	.07	.06	.05
g_6	+	.00	.02	.02	.03	.02	.02	.02	.01	.01	.01
	-	-.01	-.02	-.03	.10	-.02	-.02	-.02	-.02	-.01	-.01
g_7	+	.02	.03	.05	.07	-.07	.05	.04	.04	.03	.03
	-	-.02	-.04	-.07	.07	-.04	-.04	-.04	-.02	-.02	-.02
g_8	+	.06	.12	.18	.25	.15	.16	.16	.15	.14	.12
	-	-.15	-.13	-.11	-.05	-.13	-.09	-.07	-.05	-.04	-.03
g_9	+	-.02	-.04	-.06	.08	-.04	-.03	-.03	-.02	-.02	-.01
	-	.02	.03	.05	.06	-.08	.04	.04	.03	.03	.02
g_{12}	+	.00	.00	.00	.00	.02	.03	.03	.04	.04	.04
	-	.00	.00	.00	.00	-.02	-.03	-.03	-.04	-.04	-.04
g_{13}	+	.00	.00	.00	.00	.15	.18	.20	.21	.23	.24
	-	.00	.00	.00	.00	-.08	-.10	-.12	-.13	-.15	-.16
g_{14}	+	.00	.00	.00	.00	-.02	-.02	-.03	-.03	-.03	-.04
	-	.00	.00	.00	.00	.02	.03	.03	.03	.04	.04
g_{16}	+	.00	.01	.01	.02	.01	.01	.00	.01	.00	.00
	-	.00	-.01	-.01	-.02	-.01	-.01	-.01	.00	-.01	-.01
$H(0)$	+	.00	-.01	-.01	-.02	-.01	-.01	-.01	.00	-.01	-.01
	-	.00	.01	.01	.02	.01	.01	.01	.01	.01	.00
$V(0)$	+	.00	-.01	-.01	-.02	-.01	-.01	-.01	.00	-.01	-.01
	-	.00	.01	.01	.02	.01	.01	.01	.01	.00	.00

g_{21} controls the rate that contaminated foliage becomes decontaminated. Variation in g_{20} and g_{21}, 5 percent above and below nominal rates, does not cause significant change in P^*/P. The defoliation levels, however, did vary as the day of insecticide treatment changed (table 3-4). The difference in defoliation among treatment days increased as the initial population increased.

Discussion

In this model, tree volume growth ($\triangle V$) is assumed to be proportional to the production of photosynthate (P) less the uses of that photosynthate for other purposes (R). Although numerous other expressions describing P and R could be developed, the basic notion that $\triangle V = P - R$ holds for all living trees. Thus, this model provides a biologically based framework for the examination of the impact of many processes that affect P and R and, consequently, tree growth. As other insects that affect tree photosynthate production and the utilization of photosynthate become economically important, the determination of the impact of these insects should not require a whole new forest growth model, but rather a modification of the insect submodel, which in many cases should be limited to changes in the magnitudes of parameter values.

Besides being useful for impact assessment, a model of this kind is useful in identifying areas where research should be intensified or started, especially in programs such as the one described in this compendium. First, the model is developed, and then the sensitivity analysis is done to identify the processes and research areas that are most important. The sensitivity analysis of this model suggested that larval growth and consumption rate and phenology and growth rate of foliage are needed areas of research. These areas are still under investigation.

Summary

Robert L. Talerico

Ability to monitor and assess the gypsy moth host system and understand the overall effects of the damage it causes has greatly increased in the past few years. Detection efforts for the gypsy moth are becoming more sophisticated with the use of pheromones to attract adult males or canines to locate egg masses; quantification and mapping of gypsy moth defoliation with satellite imagery and advanced photometric methods appear operationally and economically feasible; and egg-mass surveys continue to be the backbone of all detection and evaluation methods, although many egg masses continue to be overlooked. The fixed-variable plot (FVP) method for observing egg masses has many operational and statistical advantages over the older more subjective methods for observing egg masses. A rapid reconnaissance method that uses a series of 5-minute walks to categorize the egg-mass density on an area is useful for screening many areas for more intensive examination with the FVP method or to locate potential areas for experimental or operational treatments. However, suitable operational methods to quantify many of the ecological, economic, and sociological impacts are still lacking.

The evaluation and decisionmaking process for deciding what courses of action to follow when a manager is confronted with a gypsy moth problem still falls into the realm of an art rather than a science,

Table 3-4.—*Percentage defoliation predicted by the gypsy moth submodel arranged according to the initial larval population and the number of days from hatch to the pesticide treatment*

Initial larval population$\times 10^{-5}$/ha	Percent predicted defoliation, by pesticide treatment day			
	No treatment	20	25	30
2	24.5	5.3	5.4	5.9
4	43.0	8.5	8.8	9.6
6	57.9	11.6	12.1	13.3
8	69.5	14.7	15.3	17.0
10	78.3	17.7	18.5	20.5
12	85.0	20.6	21.6	23.9
14	89.9	23.5	24.6	27.3
16	93.4	26.3	27.5	30.6
18	95.9	29.1	30.4	33.7
20	97.8	31.7	33.2	36.8

although, progress is being made in understanding the many interactions that occur. Mathematical models and computer simulation methods have been demonstrated as aids in the decisionmaking process and permit the manager to view the consequences of several actions before the fact. The decision tree technique permits the manager to list the possible alternatives and select the one method that will cost the least to implement.

Detection and evaluation methods are continually evolving through use and the development of new technology. Continued interaction between research and development technology will provide the manager with the tools for detecting and evaluating alternative strategy to cope with the gypsy moth.

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Population Dynamics

Introduction
Robert W. Campbell

Defining Population Dynamics

Most definitions of the term "population dynamics" include both the idea of changes in population density and the causes of these changes (Campbell 1967b, Clark et al. 1967, Huffaker and Messenger 1964, Krebs 1972, Nicholson 1954). Some investigators have also included population quality as an integral part of population dynamics (Waters 1969, Wellington 1957). In this publication, the scope of population dynamics includes evolving interactions that tend to modify numerical changes within the gypsy moth life system, and the literature on gypsy moth populations has been summarized around the idea that "population dynamics is the science which explains how and why the abundance of living things fluctuates" (Stark 1977). In addition, information is presented which transcends the classical scope of population dynamics. Dispersal, diapause, egg and larval development and meteorological influences were considered sufficiently important to a more thorough understanding of gypsy moth population dynamics to warrant their inclusion.

World Literature

Gypsy moth activities are of considerable economic importance in Europe, Asia, Africa, and North America. For this reason, major regional research efforts have been directed against the pest for many years and have resulted in a massive collection of literature on many aspects of the overall pest/ host/ socioeconomic control system. Because such efforts have never been coordinated, the literature base is scattered and fragmented.

Many of the publications listed at the end of this chapter are in an annotated bibliography on gypsy moth population dynamics (Campbell et al. 1978). This bibliography of 592 titles and 450 annotations probably includes most of what was known about the population dynamics of this insect through about 1974; a smaller proportion of the more recent literature is included.

Investigations involving a truly broad range of specific gypsy moth related, population-level topics have been reported in the world literature. For example, the 450 annotated papers in Campbell et al. (1978) were categorized as dealing with 125 population-level subjects. Of these, 94 categories were related directly to the insect, while 31 were more closely related to defoliation and its effects.

A recent note (Schaeffer 1978) reemphasizes major differences between gypsy moth strains. Conclusions about this life system drawn from the world literature must be made with caution.

Historical Review
Robert W. Campbell

This review is based on two concepts. First, numerical patterns of the population system provide a framework for describing the forces, events, and processes involved in determining these patterns. Then, the forces, events, and processes that are described explain the numerical patterns that have been observed.

Overall Numerical Behavior

Campbell (1975) concluded that a gypsy moth population system (or metapopulation, in the sense of Wilson (1975)) is capable of numerically bimodal behavior in North America (fig. 4–1). This system has four distinct features—two relatively stable modes (outbreak and innocuous) and two transient phases (release and decline).

First, the system can remain sparse indefinitely (fig. 4–1, innocuous mode), although processes that result in low-density stability have the least effect along the advancing front of the generally infested area. Innocuous populations in North America normally appear to range between 2 and 25,000 fourth-instar larvae per hectare. A second set of processes occasionally enables a sparse population to expand to the outbreak mode (fig. 4–1, release phase).

Once an areawide outbreak is underway, processes can sometimes maintain it for up to a decade (fig. 4–1, outbreak mode). Average outbreak densities in North America normally appear to range from 250,000 to

2,500,000 fourth-instar larvae per hectare. Individual subpopulations in areawide outbreaks range from 250 to 12,500,000 fourth-instar larvae per hectare.

Finally, there are processes that result in areawide outbreak collapse (fig. 4-1, decline phase).

In contrast to bimodal behavior, the numerical behavior of European gypsy moth populations has often been described as "cyclic," especially in southeastern Europe. Such populations tend to increase from innocuous to outbreak levels every 7 to 9 years (Dobrivojević 1963, Hadzistević and Hadzihalilović 1959, Maksimović and Politeó 1970, Maksimović et al. 1970, Vasiljević and Injac 1973). Outbreaks follow a rather regular pattern, and persist for about 3 years (Chugunin 1959, Georgijević and

Vaclav 1958). Major phases within these cycles have been described as latency, progradation, culmination, and retrogradation (Vasiljević and Injac 1973). Roughly equivalent terms are innocuous, increase, outbreak (including year of collapse), and postoutbreak.

Intergeneration Changes

The largest body of data ever collected on natural gypsy moth populations in North America was accumulated between 1911–31 by personnel of the now-defunct Melrose Highlands Gypsy Moth Laboratory. The Melrose workers established 264 circular 0.18-acre plots in 1911 and 1912 "... in dif-
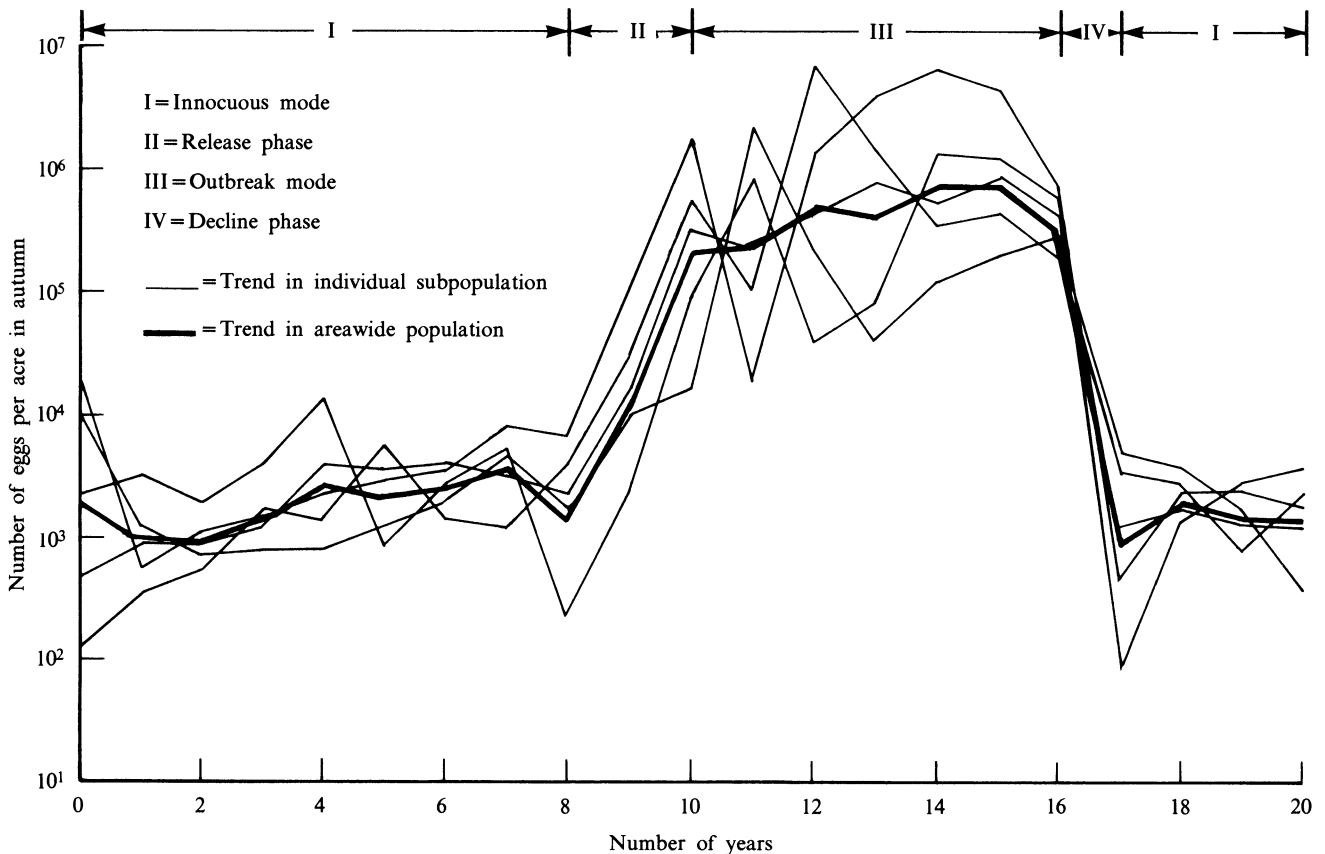
Figure 4-1.—*Numerical bimodality in a hypothetical North American gypsy moth population* (*Campbell and Sloan 1978*b).

ferent sections [of eastern New England] where the soil, weather conditions and food plants vary. ... " (Guild 1929). These plots extended from southern Massachusetts to southern Maine, and inland from the Atlantic coastline to east-central Massachusetts.

Forty-nine of the plots established in 1911 were maintained through 1931. Records from these plots are shown in figure 4-2. These populations remained high from 1911 through 1921, receded to sparse levels between 1921 and 1922, and then tended to remain sparse from 1922 until 1931.

Egg densities in nine subpopulations studied near Glenville, N.Y., between 1958 and 1963 show three major phenomena (fig. 4-3): (1) They exhibited rapid, frequently uncoordinated numerical changes from one year to the next; (2) like the Melrose populations between 1911-22, overall density remained high from 1958 through 1963; and (3) each year, a wide range in density was represented among the nine subpopulations.

Sparse subpopulations usually increased at least tenfold from year to year during the Glenville
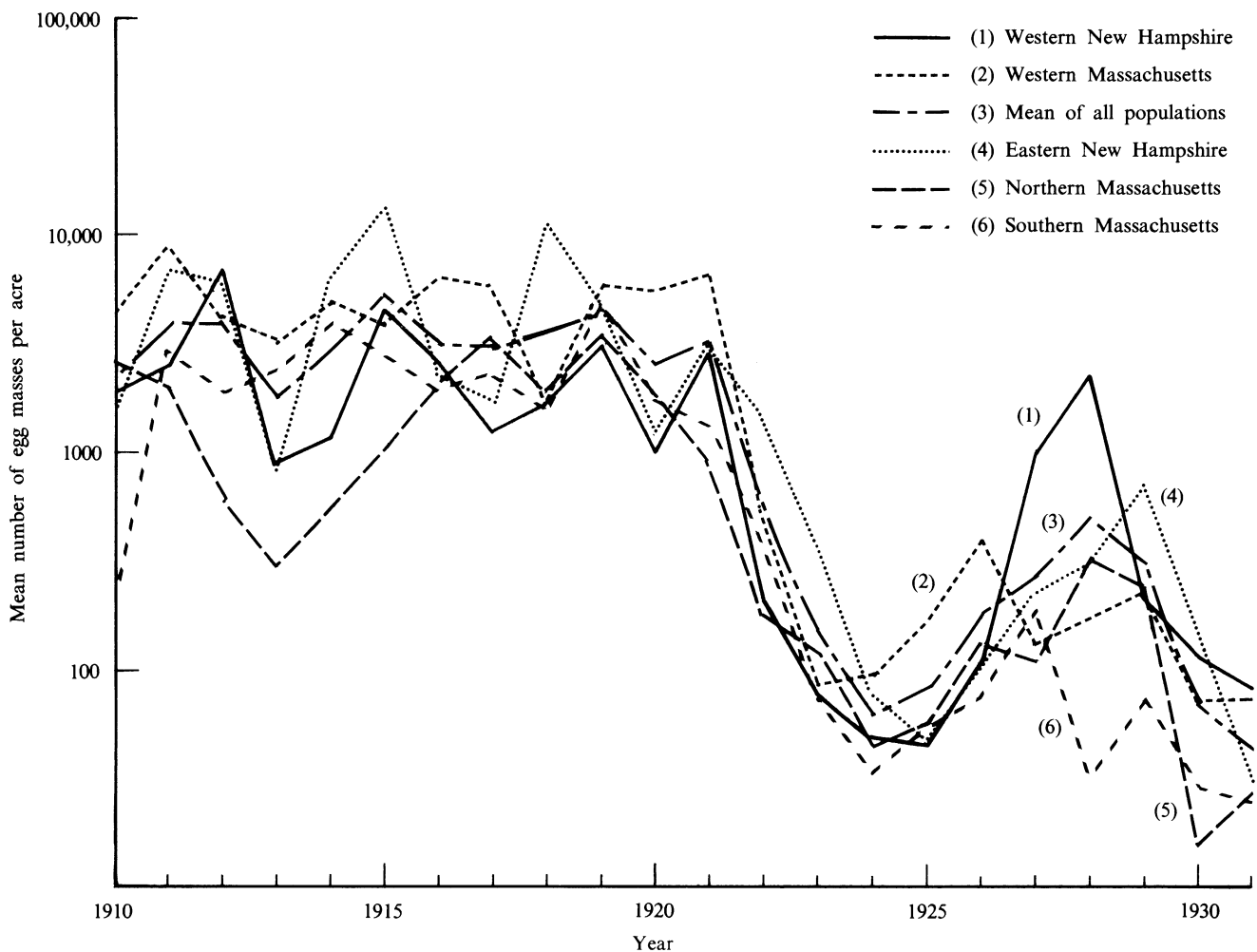
Figure 4-2.—*Gypsy moth population trends observed across 21 generations in eastern New England, 1910-31 (adapted from Campbell 1967b).*

outbreak and almost always reached outbreak densities within two or three generations. Density-dependent processes usually caused abrupt collapses within a year or two (fig. 4-3). Similar patterns were found among five sets of widely separated subpopulations (the intensive plot system, or IPS populations) during the early 1970's (Campbell and Sloan 1978b). In one extreme case, some gypsy moth related defoliation occurred for 20 consecutive years

(1925-44) in the town of Brewster (Cape Cod), Mass. (from data in Bess et al. 1947).

Little has been written about the role of variation in density on the dynamics of the individual subpopulations, but Campbell (1973) concluded that individual Melrose Highlands subpopulations may frequently have been influenced more by conditions in neighboring subpopulations than by onsite conditions. Some European authors have also referred to a
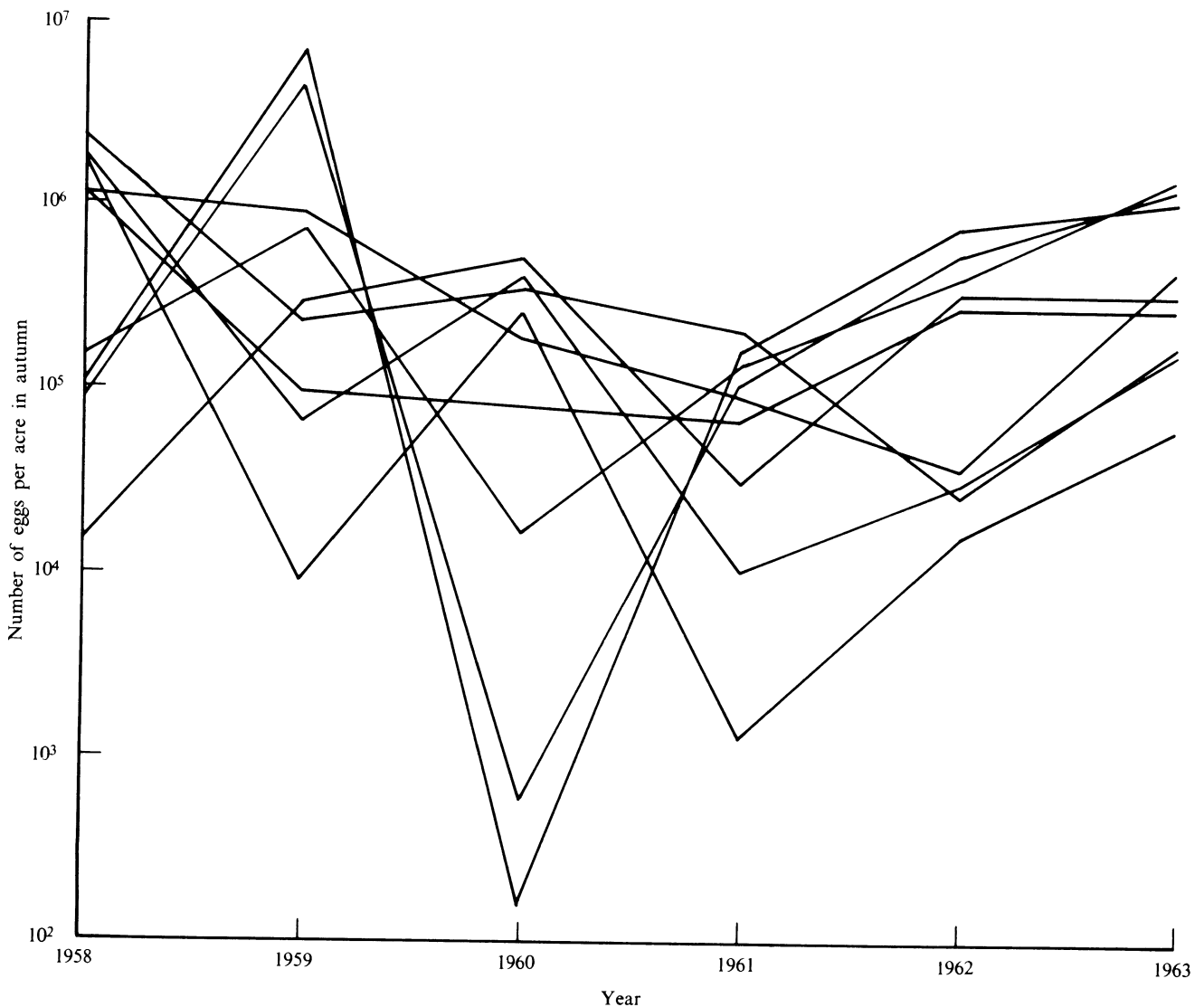
Figure 4-3.—*Number of eggs per 0.4 ha each autumn, 1958-63, in nine sites near Glenville, N.Y.* (*Campbell and Sloan 1978*b).

mixture of gypsy moth densities and trends among subpopulations in the same area (Dobrivojević 1963, Szalay-Marzsó 1957).

Many extended gypsy moth outbreaks have been reported from locations in Europe, Asia, and North Africa. Chronic outbreak conditions have been implied for gypsy moth populations on cork oak, *Quercus suber* L., in Portugal, Spain, and Morocco (Baeta Neves 1947, de Lépiney 1930, Romanyk 1965). Keremidchiev (1972) described extended outbreaks in Bulgaria, and both Khanislamov et al. (1958) and Ryvkin (1957) noted that gypsy moth outbreaks can be prolonged in the U.S.S.R. Studies by Rao (1972) on the Indian gypsy moth, *Lymantria obfuscata* Walker, suggest that populations of the insect remained at outbreak levels in southern India for at least 5 years.

In contrast, the gypsy moth population remained sparse in an area in northeastern Connecticut and the adjacent portions of Massachusetts (Eastford) between 1965-71. During these years, a subpopulation was reduced soon after it reached a substantially higher density than the average (table 4-1). A similar numerical pattern was found among the IPS populations during the year each of these latter populations collapsed (Campbell and Sloan 1978*b*).

Except for the classic work by H. A. Bess (1961, Bess et al. 1947) and the study of sparse Eastford populations by Campbell, detailed study of sparse, stable gypsy moth populations has been neglected by North American workers. Several foreign investigators, however, have reported studies on sparse, numerically stable populations (Furuta 1976, Semevsky 1973).

Apparently, noncyclic numerical behavior is common among gypsy moth populations in many locations around the world.

Intrageneration Changes

Trend in egg density from one generation to the next [(*n*) to (*n*+1)] is shown as a function of egg density at the start of generation (*n*) for both the Glenville and Eastford subpopulations in figure 4-4. Some density dependence existed; trends within each area were closely associated with density. Representative life tables for each area are presented in tables 4-2

Table 4-1. *Gypsy moth egg masses per acre (Eastford data), 1965-68*

| Site | Year egg masses deposited | | | |
	1965	1966	1967	1968
3	2	3	4	—
6	1	1	18	—
7	1	2	9	—
8	1	2	0	—
11	3	5	4	—
5	9	4	[1]14	—
9	1	2	[1]0	—
10	0	3	[1]5	—
2	6	10	8	0
4	5	5	8	1
12	5	0	1	0
1	—	39	50	3
5	—	—	[1]250	6
9	—	—	[1]250	3
10	—	—	[1]250	2
4A	—	—	[2]63	5
9A	—	—	[2]33	1

[1]250 egg masses were placed in this site in winter 1967-68.
[2]Small mammals were continuously trapped and removed from this site in summer 1967.

Source: Campbell and Sloan 1978*b*.

through 4-4. The populations represented by these tables exhibited both similarities and differences in fecundity, major mortality factors, age interval survival rates, adult sex ratios, and generation trends. Processes involved in all these phenomena are described throughout the next section.

Annual rates of increase similar to the sometimes spectacular rates shown in figure 4-4 for sparse Glenville subpopulations have been reported for European populations. Populations in Yugoslavia increased about fortyfold just before they erupted, then they increased about 270-fold "... between the preeruptional and the eruptional year. ..." (Vasić 1958).

Differences in generation trends between Glenville and Eastford can be seen from figure 4-4. Starting from common densities, generation trends in Glenville were much higher than those in Eastford.

Specifically, the contribution of each major age interval survival rate to generation differences between sparse subpopulations in the two areas is shown in figure 4-5. The following is a summary of

what this figure represents. Survival rates among first-through third-instars, fourth- through sixth-instars, and pupae were all higher in Glenville than in Eastford. As density changed, survival differences between the two areas also changed. As density increased, differences in first- through third-instar survival decreased, while differences in fourth- through sixth-instar survival increased. Differences in pupal survival were relatively constant across the common density range.

Another, major difference—the consistently higher proportion of females among adults in Glenville—arose from unequal mortality rates between the subadult male and female insects, which differed among the populations in the two areas. Processes involved in differential mortality of the sexes, variation in generation survival, and differences in survival among areas are described in the following section.
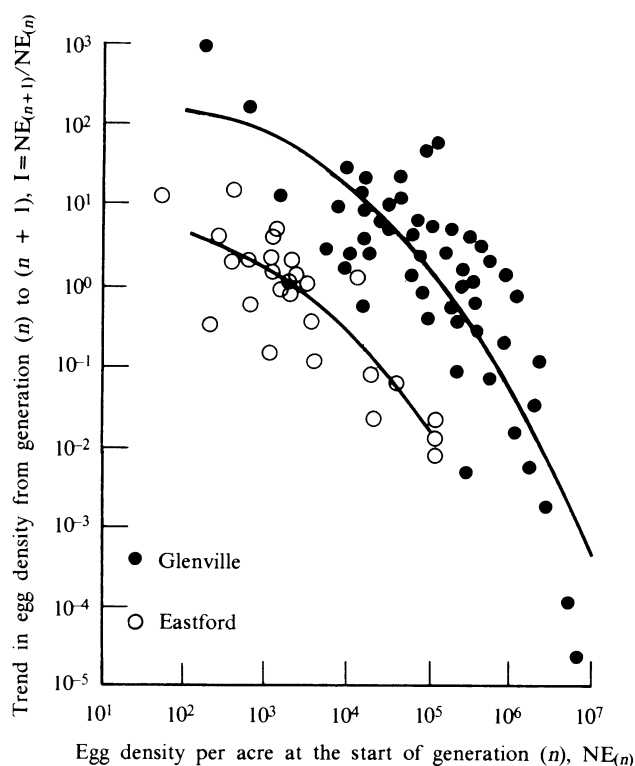
Figure 4-4. – *Relationship between trend in egg density* (I) *and egg density at the start of the generation* (NE$_n$) *(Glenville and Eastford data, 1958-64 and 1965-68) (Campbell and Sloan 1978b).*

Table 4-2.—*Life table typical of dense gypsy moth subpopulation in an areawide outbreak (Glenville data)*

Age interval (x)	Number alive at beginning of x (lx)	Factor responsible for dx (dxf)	Number dying during x (dx)	dx as percent of lx ($100qx$)
Eggs	[1]250	Parasites	50.0	20
		Other	37.5	15
		Total	87.5	35
Instars 1-3	162.5	Dispersion, etc.	113.8	70
Instars 4-6	48.7	Parasites	2.4	5
		Disease	29.2	60
		Other	12.2	25
		Total	43.8	90
Prepupae	4.9	Desiccation, etc.	0.5	10
Pupae	4.4	Parasites	1.1	25
		Disease	0.7	15
		Calosoma larvae	0.9	20
		Other	0.4	10
		Total	3.1	70
Adults	1.3	Sex ($SR = 30:70$)	0.9	70
Adult females	0.4	—	—	—
Generation	—	—	249.6	99.84

[1]Number of eggs in an average egg mass.
Source: Campbell 1969.

Processes

An important age-interval survival rate in determining generation survival during one mode may play a minor role during another. Similarly, major processes affecting innocuous and outbreak populations differ so thoroughly that the two overall modes may almost appear to be drawn from different life systems. For example, vertebrate predation is important among sparse populations and minor among dense ones, while the converse is true of disease.

For the above reason, the categorization of processes in this review into either life stages or types of processes (mortality-causing agents, food, weather, etc.) seemed both cumbersome and potentially confusing. This section describes instead the major

processes that operate during each mode or phase (innocuous mode, release phase, outbreak mode, decline phase) and concludes with a review of what appears to be an evolving interaction between the insect and its host food plants.

Innocuous Mode

According to Forbush and Fernald (1896), "No one except Trouvelot is known to have observed [the gypsy moth] during any portion of the [the first 10 years] ..." following its introduction into North America. The insect must have remained fairly innocuous during its first decade on this continent.

Nevertheless, some continue to believe that "populations of natural predators and parasitoids were absent from our forests, and gypsy moths reproduced unhindered" (Simser 1977).

Most gypsy moth populations, like those of other forest defoliators, are usually maintained at innocuous levels once the original outbreak, which often follows shortly after invasion, has subsided. Outbreaks continue to occur, but they are usually less spectacular and damaging than the original ones.

Although Forbush and Fernald (1896) stressed the importance of avian predators in gypsy moth population dynamics, they and subsequent investiga-
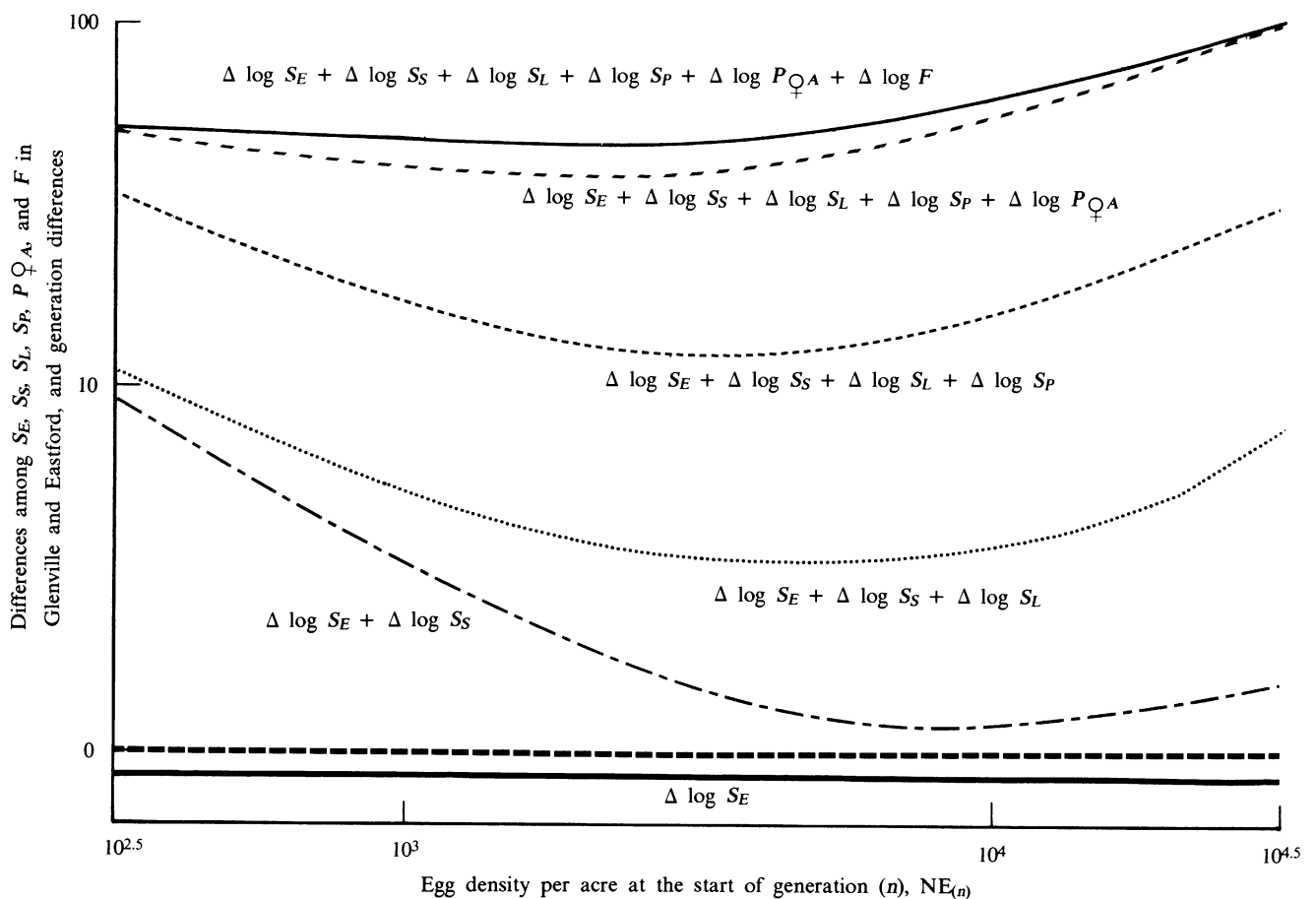

Figure 4-5.—*Differences between Glenville and Eastford in the sum of gypsy moth survival rates, as related to egg density at the start of the generation (Campbell 1976). S_E = survival rate of eggs, S_S = instars 1-3; S_L = instars 4-6; S_P = pupae; $P\female_A$ = proportion females among adults. F = fecundity.*

Table 4-3.—*Life table typical of sparse gypsy moth subpopulation in an areawide outbreak (Glenville data)*

Age interval (x)	Number alive at beginning of x (1x)	Factor responsible for dx (dxf)	Number dying during x (dx)	dx as percent of 1x (100qx)
Eggs	1450	Parasites	67.5	15
		Other	67.5	15
		Total	135.0	30
Instars 1–3	315	Dispersion, etc.	157.5	50
Instars 4–6	157.5	Parasites	7.9	5
		Disease	7.9	5
		Other	118.1	75
		Total	133.9	85
Prepupae	23.6	Desiccation, etc.	0.7	3
Pupae	22.9	Vertebrate predators	4.6	20
		Other	2.3	10
		Total	6.9	30
Adults	16.0	Sex (SR =65:35)	5.6	35
Adult females	10.4	—	—	—
Generation	—	—	439.6	97.69

[1]Number of eggs in an average egg mass.
Source: Campbell 1969.

tors also thought that the absence of effective insect parasites and predators in North America was a major factor favoring outbreaks (Burgess and Crossman 1929, Howard and Fiske 1911, Nichols 1973).

Food Quality

Various oaks, *Quercus* spp., are favored by gypsy moth larvae, and silvicultural recommendations were based on the premise that both gypsy moth densities and defoliation are determined by the proportion of favored food trees in the stand (Baker and Cline 1936, Behre 1939, Behre and Reineke 1943, Behre et al. 1936). Barbosa and Capinera (1977) and Hough and Pimentel (1978) found that gypsy moth development and fecundity were higher among insects on a diet of oak foliage than among those on a maple foliage diet.

North American studies of sparse, stable gypsy

moth populations have been concentrated in the largely oak forests of northeastern Connecticut and adjacent Massachusetts. Although these forests can support a massive outbreak (Anderson and Gould 1974), populations there have generally remained sparse for more than 50 years. Several writers have commented on this stable population, usually noting that the processes involved were poorly understood (Behre et al. 1936, Brown and Sheals 1944, Friend 1945, Turner 1963).

Following studies in the above area, Bess et al. (1947) disputed both the favored food theory and, by implication at least, the notion that the absence of effective insect parasites and predators is an important
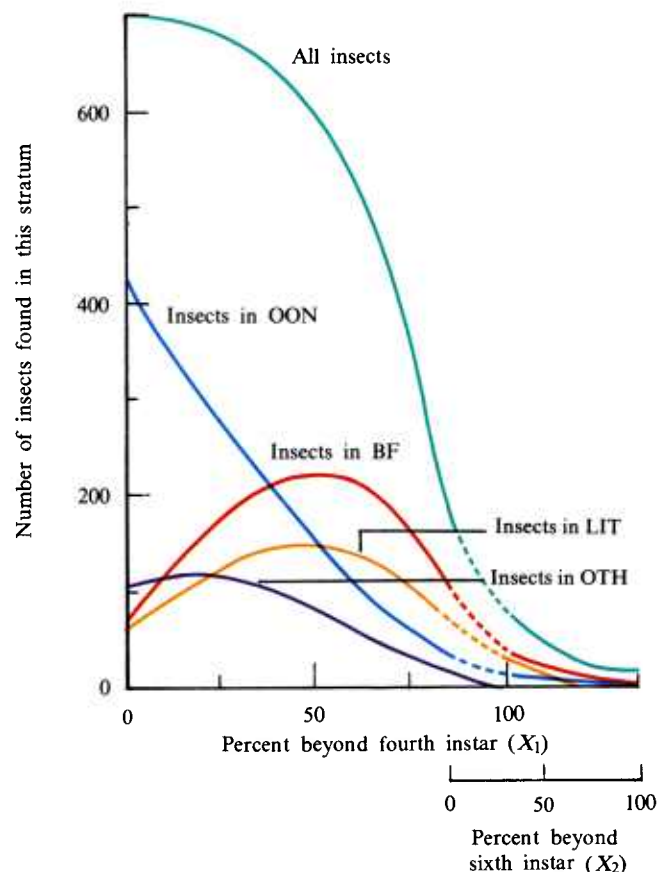

Figure 4-6.—*Insects found within various environmental strata as a function of insect stage (Eastford data) (Campbell et al. 1975a). BF= bark flaps; LIT= litter; OON= other locations on oak; OTH= other.*

Table 4-4.—*Life table typical of sparse gypsy moth subpopulation when areawide population is innocuous (Eastford data)*

Age interval (x)	Number alive at beginning of x (lx)	Factor responsible for dx (dxf)	Number dying during x (dx)	dx as percent of lx ($100qx$)
Eggs	[1]450	Parasites	67.5	15
		Other	67.5	15
		Total	135.0	30
Instars 1–3	315	Dispersion, etc.	72.5	23
Instars 4–6	242.5	Deer mice	12.1	5
		Parasites and disease	12.1	5
		Other	203.7	84
		Total	227.9	94
Prepupae	14.6	Predators, etc.	2.9	20
Pupae	11.7	Vertebrate predators	8.2	70
		Other	2.1	18
		Total	10.3	88
Adults	1.4	Sex (SR=30:70)	1.0	70
Adult females	0.4	—	—	—
Generation	—	—	449.6	99.93

[1]Number of eggs in an average egg mass.
Source: Campbell 1969.

factor favoring outbreaks in North America. They noted that "the history of this insect in the oak forest of Connecticut and western Massachusetts shows that food supply does not normally limit the abundance of the moth in these areas." They felt that "... large larvae survived in much greater numbers where they molted and rested above the forest floor, rather than in the litter ...," and, emphasized the importance of insectivorous small mammals in habitats where the insects spend the day in the litter. Many species of North American small mammals will readily eat gypsy moth larvae or pupae, or both (Smith and Campbell 1978).

Recent results only partially support the favored food theory. Contrary to theory, there was little difference in the Melrose Highlands plots between the

Table 4-5.—*Egg masses found in the fall of 1971 in treated 0.4-ha plots where 100 egg masses had been placed in each plot in the spring of 1971 (Eastford data)*

Forest	Mammals removed	Birds excluded	Mammals removed and birds excluded	Control
Brimfield 1	124	7	357	14
Brimfield 2	187	28	71	[1]101
Nipmuck 1	215	10	186	35
Nipmuck 2	44	0	129	21
Harold Parker	8	0	23	3
Willowdale	1	4	7	0
Mean	96.5±[2]37.7	8.2±4.3	128.8±53.1	14.6±6.3

[1]Unfortunately, this control plot was situated in an area that had recently been burned over. Too late it was found that this burn had resulted in the decline or death of many trees. These trees had produced many bark-flaps, which provided secure resting locations for many insects. This check plot was therefore not included in calculating average survival in control plots.
[2]Standard error.
Source: Campbell and Sloan 1977*b*.

stable state distribution of egg-mass densities in favored food stands and in poor food stands (Campbell 1974*a*). However, the percentage of favored food in the overstory was clearly related to subsequent defoliation (Campbell 1974*b*).

The Eastford Study

From 1965 through 1971, a new study was conducted on gypsy moth population dynamics near Eastford, the same approximate area studied earlier by Bess.

When population density was low, a low dispersal rate was apparent among newly hatched larvae, and most established larvae were found near egg masses. Until the insects molted into the third instar, most of them remained on or near the foliage.

Sometime before they reached the fourth instar, the insects began to rest during daylight hours in locations other than the foliage. At first, most of them rested on tree boles, but they eventually found resting locations protected from both precipitation and direct light. These locations were usually under bark flaps, if available; otherwise most of the insects rested and

pupated in the litter (fig. 4–6). Campbell and Sloan (1976) postulated that this behavior evolved in response to high mortality from natural enemies in the trees.

Survival during instars 4–6 in Eastford was closely related to larval density (fig. 4–7). Average survival was reduced from about 22 percent where 250 larvae per hectare had hatched, to about 2 percent where 100,000 had hatched. At all densities, larvae that rested during the day in bark flaps were more likely to survive than those that rested in the litter (fig. 4–8).

Although about 90 percent of the late-instar larvae died in Eastford, few were killed by parasites or disease; average mortality from these sources was only 7.5 percent. It was concluded that most were eaten by density-dependent predators that forage in the litter. The results suggested that birds might be important (Campbell et al. 1977).

By the time the insects in Eastford pupated, about 90 percent were either beneath bark flaps or in the litter. Male larvae were more likely than females to pupate under bark flaps; pupal survival was higher under bark flaps (Campbell et al. 1975b).

Vertebrate predators killed about 70 percent of the Eastford pupae; white-footed mice, *Peromyscus leucopus* Raf., were the most important predators.
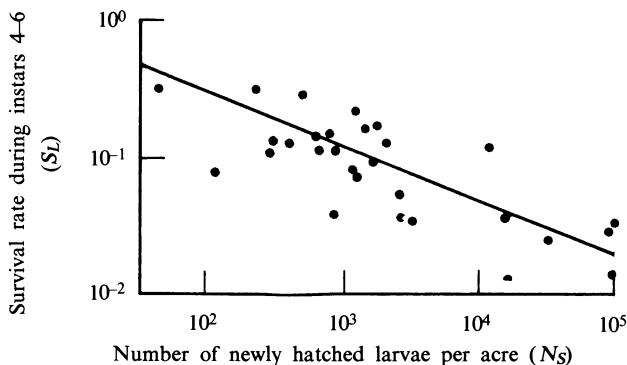
Pupae in the litter were more likely to be preyed upon than those in other locations; female pupae were more likely than males to be killed (Campbell and Sloan 1976).

By 1971, the hypothesis had been developed that numerical stability among the sparse Eastford populations was caused primarily by the combined effects of predaceous birds, which were a primary source of mortality during the fifth- and sixth-instars, and small mammals, which were the primary cause of mortality among pupae.

To test the above hypothesis a series of four 0.4-ha plots, replicated 6 times, was established. One hundred egg masses were placed within each plot. One plot was used as a control. Small mammals were removed from the second. Burlap strips protected by poultry netting were placed around each tree bole in the third plot, to foil predaceous birds. Both small mammal removal and predaceous bird devices were used in the fourth plot. Results showed conclusively that mammals played a major role in the dynamics of these populations (table 4–5) and suggest that removal of small mammals plus exclusion of birds had a more profound effect than did removal of small mammals alone (Campbell and Sloan 1977b).

Egg-mass densities were actually lower in plots

Figure 4–7.—*Relationship between number of newly hatched larvae per 0.4 ha* (N$_s$) *and subsequent survival rate during the fourth through sixth instars.* (S$_L$) (*Eastford data from 1965-68*) (*Campbell et al. 1977*).
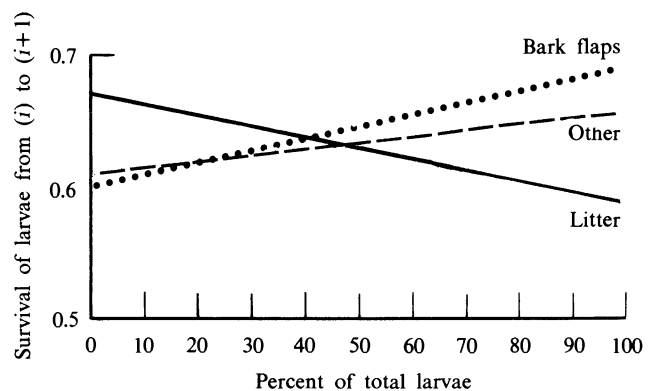

Figure 4–8.—*Relationship between survival of larvae across a short interval of time and the percentage of the total larvae found in litter, in bark flaps, and in other resting locations* (*Eastford data*) (*Campbell et al. 1977*).

where birds had been controlled than where nothing had been intentionally altered; small mammals in the former plots ultimately found and consumed nearly all insects resting beneath the burlap bands. These results emphasize two concepts. First, while prey location is important, these predators are capable of learning where prey insects are located. Second, vertebrate predators, collectively, may be necessary to contain a sparse gypsy moth population, but either birds or small mammals alone may be sufficient (Campbell and Sloan 1977b).

Results in Campbell and Sloan (1977b) also emphasized the importance of selective predation on female pupae by mammals. About half the pupae were females in the Eastford area. Only about one-fourth of the adults were females, because of this selective predation.

Finally, when density fecundity data from Eastford were compared with similar data from an extended outbreak area near Glenville, N.Y., eight of the nine average fecundity rates observed in Eastford were lower than those found in Glenville at similar densities. This situation is worthy of further study.

Other Studies

References to vertebrate predation on the gypsy moth are fairly common in the Eurasian literature but almost all refer to birds. Bruns (1960) concluded that birds can remove substantial proportions of low-density insect pest populations, and Turcek (1950) noted that birds probably play a role in maintaining gypsy moth populations at low levels. Dobrivojević (1963) noted that both *Oriolus galbula* L. and *Sitta europa* L. are important predators of larvae and eggs. Luhl and Watzek (1977) concluded that "5.6 successful broods [of cavity-nesting birds] pro [sic] hectare may suffice to protect the stand against caterpillar outbreak [including the gypsy moth]." Strokov (1956) noted that predaceous birds can be important in gypsy moth population dynamics in the Soviet Union, and avian predators formed the biological core of an integrated control system being developed against several forest defoliators in the U.S.S.R. in 1975 (U.S.-U.S.S.R. 1975). Furuta (1976) concluded that birds in Japan were the most

important factor in maintaining gypsy moth populations at low levels.

References to predation on the gypsy moth by small mammals are all but absent from Eurasian literature. Rotschild (1958), however, found the remains of both gypsy moth larvae and pupae in the stomachs of *Apodemus flavicollis, A. sylvaticus,* and *Dyromys nitedula.* He noted that the stomachs of these mice were often filled exclusively with remains of these insects. Semevsky (1973) concluded that predators play the most important role in the dynamics of sparse gypsy moth populations, but he did not identify the predators.

Parasites have often been assigned a minor role in the dynamics of sparse gypsy moth populations in Eurasia (Furuta 1976, Komarek 1950, Patočka and Čapek 1971, Semevsky 1973). Others believe that parasites can play a major role during this phase (Gyorfi 1961, Maksimović et al. 1972). Nematodes play an undefined role among European populations (Drea et al. 1977).

Relatively little is known about the role of invertebrate predators in gypsy moth population dynamics; most of the literature is on the beetle *Calosoma sycophanta* L. Patočka and Čapek (1971) and Vasić (1972) state that this species is rare when the gypsy moth population is sparse.

Disease apparently plays a minor role among sparse gypsy moth populations (Vasiljević and Injac 1973), but adverse weather or climate may be important in maintaining such populations at innocuous levels (Janković 1956, Maksimović 1953).

Release Phase

Outbreak Foci

A major problem in trying to understand gypsy moth population dynamics is tracing an outbreak back to its origin. Although dispersal of young larvae undoubtedly plays a major role in the maintenance and spread of outbreaks, it probably plays a relatively minor role in their initiation (Campbell 1976). Except for the edge of the general infestation, even major outbreaks sometimes appear to have originated in outbreak foci—relatively small and discrete locations.

Bess et al. (1947) characterized sites susceptible to defoliation by the gypsy moth as "... light sandy or gravelly soils; a history of repeated fires; stand canopies covering less than half the ground; short, scrubby timber; and a ground cover in which certain plants are abundant, notably blueberry, sweetfern, and bracken. Leaf litter is sparse or absent; and in the more open types, especially where grazed, mats of grass or *Carex* may be frequent. Dominant in the forest are gray birch, aspen or oaks characteristic of fairly dry sites, particularly scarlet oak, white oak, and black oak. An abundance of white pine and oak reproduction in the understory is frequently characteristic."

Secure Resting Locations

Bess et al. (1947) thought that sparse gypsy moth populations would tend to increase in woodlands where the insects rested and pupated in places other than the litter. This hypothesis was recently validated (Campbell and Sloan 1976, 1977b, Campbell et al. 1975a, 1977).

Another hypothesis states that sparse populations tend to be released when they are near human activities (Campbell et al. 1976). The woodland edge had already been implicated by Eurasian investigators as containing likely foci for outbreaks (Golubev and Semevsky 1969, Kokhmanyuk 1965, Patočka 1973). Campbell et al. (1976) found that egg-mass density was 10 times higher along the forest edge than within the forest, at least in places where overall egg-mass density was less than 125 egg masses per hectare (table 4-6). Manmade objects along the forest edge contained about one-half of the total egg masses found at low densities. Rough, dry manmade objects that were protected from light contained the most.

Collectively, the results led to the hypothesis that some outbreaks originate in sites where naturally occurring, protected locations (for example, bark flaps) or their equivalents (manmade objects) provide abundant resting locations above the forest floor. Results of a recent test showed not only that trees with manmade objects had several times as many egg masses as control trees, but also that trees with

Table 4-6.—*Gypsy moth egg-mass density in woodland plots and in plots along the woodland edge*

Range in egg-mass density per acre under woodland conditions	No. of home sites per year in stratum	Egg masses per acre		Edge plots divided by woodland plots
		Wood-land plots	Plots along woodland edge	
≤50	90	8.6	80.9	9.5
51 to 200	35	74.0	459.1	6.2
201 to 500	32	353.4	704.4	2.0
501 to 1000	22	643.6	674.6	1.1
>1000	26	1,935.4	2,198.8	1.1
Total	205	386.1	575.2	1.5

Source: Campbell et al. 1976.

manmade objects along the forest edge tended to have higher egg-mass densities than similar trees in the forest proper (table 4-7).

Campbell and Sloan (1977c) concluded that gypsy moth outbreaks can start in sites with many protected resting locations. They characterized such sites as follows: "Bark flaps were ... used ... most ... in the woodland sites ... and [manmade objects] ... served the same function along the woodland edge.... Tree forms found among poorly stocked stands such as those growing on dry, rocky ridges or excessively drained sands tend to provide abundant sheltered locations (Bess et al. 1947, Houston 1975). Remnant "wolf-trees" may also represent outbreak foci.... And ice storms, which often kill portions of the tree crown, can create ideal locations within the crowns for larval and pupal survival...."

Houston and Valentine (1977) concluded that "forests rich in ... both favored ... food and ... aboveground resting and hiding places ... would support higher populations...."

Predator Failure

Even sparse populations accessible to predators may become outbreaks. In one recent instance, synchronous, rapid increases in density were observed among six subpopulations in the vicinity of Whitehall,

Table 4–7.—*Gypsy moth egg-mass density on trees with man-made objects and on control trees, in plots along woodland edge and within woodland*

Study area	All control trees (±standard error)	Trees with manmade objects woodland edge	Trees with manmade objects within woodland
Connecticut:			
Woodbury	0.03±0.03	0.60±0.36	0.25±0.10
Southbury A	0.22±0.07	2.06±0.45	2.31±0.55
Southbury B	0.25±0.08	1.13±0.26	0.65±0.21
Southbury C	0.36±0.12	1.50±0.32	1.11±0.24
Washington A	0.42±0.09	2.63±0.49	1.47±0.22
Washington B	0.47±0.11	2.94±0.60	1.50±0.30
New York:			
Lake Mohonk	0.43±0.14	4.03±0.75	1.85±0.59
New Jersey:			
Pine Hill	0.34±0.09	7.46±1.96	6.42±1.25
Horn	1.27±0.25	5.83±1.14	5.33±1.61
Clover	5.46±0.91	11.08±1.46	8.88±1.61
Harbourton	19.53±3.61	26.16±2.68	27.79±4.53

Source: Campbell and Sloan 1977c.

N.Y. (fig. 4–9). Unfortunately, detailed observations were not made during years of increase.

Other Release Mechanisms

Stand composition has been described as important in gypsy moth population buildup (Clement and Munro 1917); Behre (1939) felt that outbreaks are unlikely where favored-food trees are less than 50 percent of the stand. In Europe, Patočka and Čapek (1971) noted that outbreak foci are located in areas of high-quality food.

Population release is thought by some to be related to meteorological conditions, which may disrupt the insect's control system by stressing host trees and reducing predation and disease (Vasić 1958). Key weather events may include either a series of cold winters and hot, dry summers (Benkevich 1964, Khanislamov et al. 1962), or simply the lack of cold weather in mid-May (Patočka and Čapek 1971). In considering causal pathways, however, Khanislamov and Girfanova (1964) have shown that variations in weather may have more drastic effects on selected

natural enemies of the gypsy moth than on the pest itself.

Several authors have commented on the possible role of nutrient imbalances in gypsy moth population release (Buttner 1961, Merker 1960, Patočka 1973). Little work has been done in this area in North America.

Although Sisojević (1975) found that percentage parasitism was highest in Yugoslavia at intermediate host densities, Semevsky (1973) concluded that percentage parasitism does not increase substantially as host density increases. Similarly, both invertebrate predators and disease usually play minor roles during population increases in Europe (Khanislamov et al. 1962, Vasić 1972, Vasijević and Injac 1973).

Leonard (1971a) suggested that "... population flushes are a necessary event in the biology of the gypsy moth to bring about dispersal and genic mixture." He postulated that a qualitative change, which is governed by density, acts as a self-regulating mechanism; specifically, adults that were stressed as larvae lay small, energy-deficient eggs. Such eggs produce a high incidence of additional molting types, which according to Leonard(1971a) are more likely to be dispersed by the wind. Recently, several authors have disputed elements of Leonard's hypothesis. Capinera and Barbosa (1976) determined that larger larvae were *more* likely than smaller ones to undergo more than one dispersal. Also, Campbell (1978a) observed a pattern of density and rate of development among newly hatched larvae opposite to the pattern postulated by Leonard (1968a).

Turner (1961) postulated that a sparse gypsy moth population may "... increase very slowly until the individual inbreds are mixed to provide for crossbreeding," but he did not provide empirical data on the gypsy moth to support his hypothesis.

Vasić (1972) postulated that "... hybridization ... could ... reduce or possibly eliminate ... diapause.... " Following a series of laboratory trials, Vasić concluded that "... diapause was shortened little by little, and in the last three generations, it lasted only 7–10 days"(Vasić 1976). Later, Hoy(1977, 1978) reported similar results from laboratory trials with the North American strain.

Richardson et al. (1978) concluded that "some egg characteristics were meaningful in concert as indicators of [population] quality."

Combinations of events often have a decisive influence in the initiation of outbreaks. Although Bess et al. (1947) noted that land abuses (fires, repeated clearcutting, overgrazing), drought, and blowdown all contribute to favorable conditions for outbreaks, they also believed that outbreaks are actually caused by the interaction of weather, parasites, predators, and other factors. Mattson and Addy (1975) also stressed that combinations of events may tend to increase host food quality and decrease host resistance.
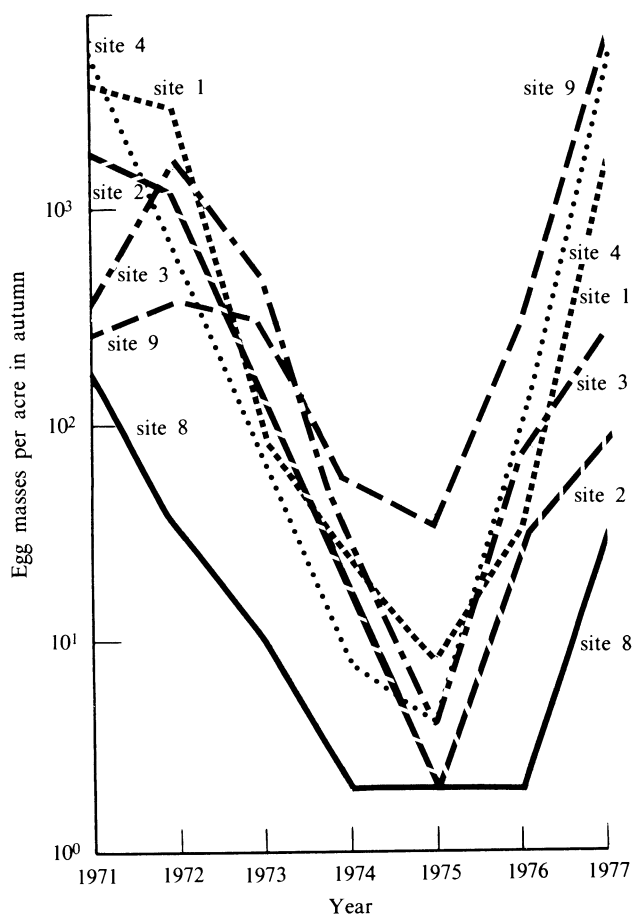

Figure 4-9. —Number of egg masses per 0.4 ha each autumn 1971-77 in six sites near Whitehall, N.Y.

In Europe, most adult insects are likely to be females during years that precede an outbreak (Keremidchiev 1972, Vasić 1950). About 60 percent of the adults were females in sparse subpopulations when the Glenville population was in the outbreak mode (Campbell 1976).

New Populations

Gypsy moth populations in North America have shown a tendency toward explosive increase near the advancing front of the generally infested area. In this area, therefore, either the processes that lead toward numerical stability at low levels are relatively ineffective, or the processes that lead toward outbreaks are exceptionally effective, or both.

In part, the above situation may reflect the time required by some introduced enemies for acclimation to new conditions. Some native North American enemies may also require acclimation to the gypsy moth. While many of these processes are unknown, Doane (1976) has implied that the nuclear polyhedrosis virus (NPV) *should* exhibit a time lag before increasing in recently invaded areas. Also, differential loss rates among favored-food and less-favored trees tend to result in stands less susceptible to defoliation than the original stands.

Recent results suggest that fecundity may be exceptionally high near the advancing front of the generally infested area. When density/ fecundity data from populations in newly infested areas in New Jersey were compared with similar data from Glenville, the probability was less than 0.05 that the two data sets were drawn from the same underlying population. Vasić and Janković (1958, 1960) in Yugoslavia have also suggested that variations in fecundity may affect population release. High fecundity in newly infested areas could also be the result, at least in part, of the high food quality that may be characteristic of many unstressed plants.

Outbreak Mode

Most investigators have focused their attention during outbreaks on individual subpopulations, which usually collapse after a relatively brief interval.

For this reason, little has been written about the processes that tend to maintain an areawide outbreak.

Variable Densities

Results described in both Campbell (1976) and Campbell et al. (1977) led to the hypothesis that intrapopulation processes tend to maintain an areawide outbreak from year to year, especially where a wide range of densities is represented.

Relationships between areawide trends in density and both density at the start of year (n) and the standard deviation in the logarithm of this density (σ) are shown in figure 4-10. The relationships shown in this figure may leave the erroneous impression that areawide populations almost always decrease. These relationships are based exclusively on populations that were already dense (Campbell and Sloan 1978a). Generally, such outbreak populations can only persist or decline.

Trends in egg density tended to decrease when σ was held constant at 0.6, its approximate mean value, but drastic declines did not occur until density approached 2.5 million eggs per hectare. Trend was optimal at about 375,000 eggs per hectare. Trend was drastically altered as a consequence of changes in σ. Rapid decreases could be expected as σ approached zero, and increases could be expected when σ was large.

These results support an earlier premise (Campbell 1973a) that individual subpopulations are sometimes influenced more by conditions within nearby subpopulations than by onsite conditions. They strengthen the hypothesis that dispersal processes involving either newly hatched larvae or opportunistic predators, or both, are important in the maintenance of an areawide outbreak. They also support the premise that effective intrapopulation dispersal occurs only where a wide range of densities is represented.

Possible Adaptations

The gypsy moth has several attributes that appear to represent adaptations to a numerically bimodal

Table 4-8.—*Estimated larval density and time of gypsy moth pupation (Glenville data),1959 and 1960*

Site	Date	Insects sampled	Percent pupae	Time of pupation (rank)	Fourth-instar larvae per acre	Larval density (rank)
			1959			
Wet site 2	June 25	190	71.1	1	524,807	1
Dry site 1	June 26[1]	230	58.6	2	112,202	4
Dry site 2	July 7	315	97.1	3	135,896	3
Wet site 3	July 7	26	92.3	4	165,959	2
Med site 4	July 7	186	86.5	5	79,433	6
Wet site 1	July 7	92	84.8	6	81,283	5
Dry site 3	July 7	305	68.8	7	75,866	7
Med site 1	July 7	190	47.4	8	54,954	8
Med site 2	July 7	95	4.2	9	8,710	9
			1960			
Dry site 3	June 24	17	58.8	1	1,862,087	1
Med site 1	June 24	52	42.3	2	1,174,898	3
Med site 3	June 24	108	38.0	3	1,202,264	2
Wet site 2	June 24	161	32.2	4	141,254	4
Med site 2	June 24	230	19.1	5	100,000	6
Dry site 1	June 24[2]	218	6.0	6	128,825	5
Med site 4	July 1	241	27.4	7	75,866	7
Dry site 2	July 9	160	46.9	8	32,359	9
Wet site 3	July 9	54	33.3	9	23,442	10
Wet site 1	July 9	66	30.3	10	43,652	8

[1]No pupae were found in any other site in 1959 until after this date.
[2]No pupae were found in any other site in 1960 until after this date.
Source: Campbell 1978a.

way of life. For example, the rate of larval development is directly related to larval density. Differences among subpopulations in Glenville were minor shortly after the insects hatched, but they increased throughout the larval interval and culminated when the insects in some subpopulations pupated 3 weeks ahead of those in others (table 4-8). This trait usually insures that a few insects will pupate in even the most dense subpopulation before the rest are overcome by overpopulation phenomena such as disease and starvation.

The average number of eggs per egg mass at the end of a generation is an excellent indicator of larval density during the generation—at least across a broad range in densities. Reductions in fecundity are evident

even below levels where significant defoliation takes place (fig. 4-11). Such reductions tend to dampen rates of increase, and thus dampen the effects of over-population phenomena, as the insects reach higher densities.

Both the ability of the larger larvae to use foliage of species on which the smaller insects would starve (Mosher 1915) and their apparent ability to alter food-getting patterns from one host species toward another as density increases (Campbell and Sloan 1977a) may represent important adaptations in situations where favored food has run out.

Finally, although gypsy moth larvae in sparse populations undergo a sharp behavioral shift during the third instar, this trait disappears completely at sufficiently high densities (Campbell 1974c). Because dense aggregations would increase the rate of spread of infectious agents, it is logical to suppose that natural selection works against individuals in dense populations that are prone to form such aggregations (Campbell 1978a).
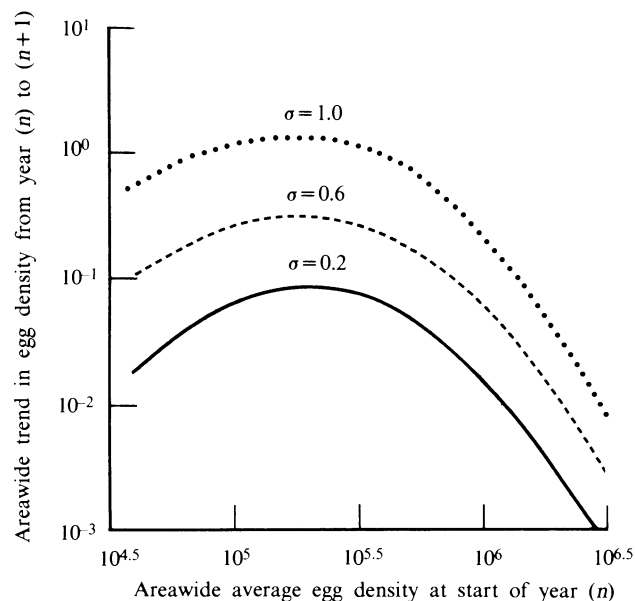
Other Studies

Turcek (1948) noted that densities of certain birds doubled or tripled during a large outbreak in South Slovakia. He also noted that predation from this source is of little value during outbreaks (Turcek 1950). It is possible that the activity of these birds actually tends to maintain outbreaks.

Khanislamov and Girfanova (1964) describe several abrupt, weather-induced shifts in natural enemies of the gypsy moth in the U.S.S.R. Depending on their direction and magnitude, such shifts might either prolong or terminate outbreaks.

Decline Phase

Most investigations of gypsy moth population dynamics around the world have focused on outbreaks. Many investigators have commented on possible reasons for population decline. Although the actual processes responsible for such declines have not often been clearly identified, dense gypsy moth

Figure 4-10.—Expected areawide trends in egg density from year n to n+1, as functions at year n of areawide egg density per 0.4 ha and standard deviation in egg density (Glenville, N.Y., and intensive plot system data) (Campbell and Sloan 1978a).
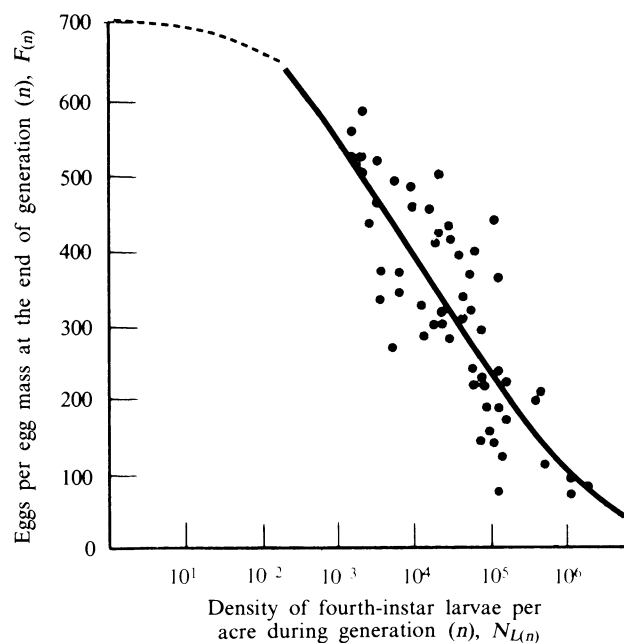

Figure 4-11.—Relationship between number of fourth instars per 0.4 ha ($N_{L(n)}$) and the average number of eggs per egg mass at the end of the generation ($F_{(n)}$) (Glenville data, 1958-63) (Campbell 1978).

populations seem to exhibit equivalent overpopulation phenomena regardless of where they occur.

Parasites and Predators

Efforts in North America to control gypsy moth populations through the introduction of foreign insect enemies constitute one of the largest undertakings of its kind (Brown 1961, DeBach 1974, Hoy 1976). Many authors have suggested that these organisms may play a major role in the natural regulation of gypsy moth numbers (Burgess and Crossman 1929, Dowden 1962, Moulding 1977, N.J. Div. Plant Industry 1974). Recent studies, however, suggest that introduced parasites generally play a minor role in the dynamics of dense North American gypsy moth populations (Barbosa et al. 1975, Campbell 1967b, N.Y. Department of Environmental Conservation 1976, Reardon 1976, Tigner 1974). Reardon, for example, who collected and reared more than 165,000 larvae and pupae from dense populations in New York, Massachusetts, and New Jersey during 1972 and 1973, concluded that parasites "... did not remove a significant proportion of the host population and did not function to limit the rate of increase of the gypsy moth...."

Regarding further parasite introduction, Barbosa (1977) suggests that "multiple introductions of both r and K strategists should be undertaken." Zwölfer (1970), however, urges that "... biological control operations should proceed cautiously with the parasite species being introduced in a predetermined sequence."

Although several species of native North American ichneumonids may sting many gypsy moth pupae in dense populations, these stung insects are unlikely to produce ichneumonid offspring (table 4–9). Rather, many of the doomed pupae may serve as a food source for scavenging sarcophagids (Campbell 1963a).

Campbell (1974c) mentioned one instance where the predaceous ground beetle Calosoma sycophanta caused heavy gypsy moth mortality. In this instance, outbreak conditions were maintained for several consecutive years. Similarly, several Eurasian investi-

gators have noted that both parasites and C. sycophanta are most likely to affect population decline where outbreaks have persisted (Chugunin 1959, Grisson 1955, Keremidchiev 1972, Vasić and Salatic 1959). Several investigators, however, state that both parasites and predators play only a minor role in terminating outbreaks in Eurasia (Komarek 1950, Nolte 1940, Semevsky 1973). Nolte (1940) suggested that C. sycophanta might be more effective in North America than in Europe.

In general, vertebrate predators appear to play a trivial role in the decline of gypsy moth outbreaks, except possibly as vectors of infectious disease (Lautenschlager and Podgwaite 1977).

Except where outbreak conditions have been maintained for several consecutive years, it seems safe to conclude that parasites and predators usually play a minor role in the decline of gypsy moth outbreaks.

Overpopulation Phenomena

Areawide population collapse appears to result primarily from the widespread occurrence of overpopulation phenomena, principally disease, reduced fecundity, and starvation.

Many authors agree that disease plays a crucial role in the decline of gypsy moth outbreaks (Bess et al. 1947, Campbell 1963b, Chugunin 1959, Doane 1970a, Dobrivojević 1963, Fiske 1913, Kaya 1976, Kolybin and Zelinskaya 1971, Komarek 1950, Patočka and Capek 1971, Semevsky 1973, Szalay-Marzsó 1957, Vasić 1958, Vasić and Janković 1958, Vasiljević 1959, Vasiljevic and Injac 1973). Disease incidence is almost always low among sparse populations, but sweeping epizootics may decimate high-density ones. Disease is likely to be more effective in wet sites than in dry or medium ones (fig. 4–12). Disease is also likely to be higher in aspen (Populus spp.) stands than in oak-hickory (Quercus-Carya) (Bess 1961). Mihalache and Pîrvescu (1977) concluded that food quality, rather than food failure, is most important in determining the intensity of an epizootic.

Major components of infectious disease in North America include both NPV (Doane 1970a, Kaya 1976) and pathogenic bacteria (Doane 1970b,

Podgwaite and Campbell 1972, Podgwaite and Cosenza 1966, 1976b). In Europe, several authors cite both polyhedrosis and microsporidiosis as playing major roles during outbreaks (Kolybin and Zelinskaya 1971, Patočka and Čapek 1971). Magnoler (1970) reported different susceptibilities among gypsy moth larvae to both nuclear- and cytoplasmic-polyhedrosis viruses from various sources.

Both noninfectious disease and combinations involving several agents can play a major role in gypsy moth population dynamics. Comparative studies on the components of disease in both dense (Old Lyme, Conn.) and sparse (Eastford) gypsy moth populations revealed that many of the diseased insects had died from unknown causes. Many insects were placed in an "undetermined" category; their status did not provide clear-cut evidence for cause of death (table 4-10). In Glenville, polyhedrosis accounted for about one-half the total larval disease loss, up to about 40 percent. Above this level, "Virus polyhedra continued to be present within nearly all dead larvae, but there were so few polyhedra present that virus could not have been the only agency involved in causing this additional mortality" (Campbell 1963b).
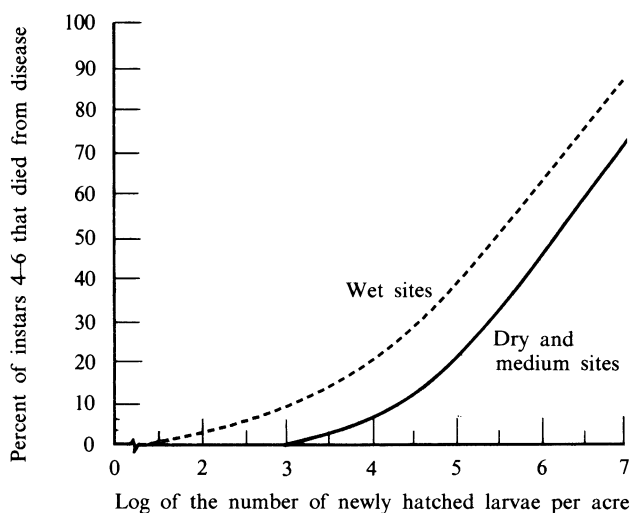
Table 4-9.—*Number of gypsy moth pupae stung by ichneumonids and number producing ichneumonid offspring (Glenville data), 1958-60*

Ichneumonid species	Number stung	Number producing ichneumonids
Theronia atalantae Cresson	16	4
Pimpla pedalis Cresson	20	1
Itoplectis conquisitor (Say)	47	0
Total	83	5

Source: Campbell 1963a.

Recent studies suggest that both dermestids and parasites can contribute to the level of virus activity (Mihalache et al. 1977, Reardon and Podgwaite 1976, Raimo et al. 1977).

Outbreaks have tended to decline during years when June precipitation was high (Campbell 1967b, Campbell and Sloan 1978a). Since Wallis (1957, 1960) has shown a positive correlation between humidity and larval disease, it seems reasonable to suppose that high June precipitation may result in an exceptionally high incidence of disease.

Starvation and reduced fecundity also play a major role in the decline of dense gypsy moth populations (Bess et al. 1947, Campbell 1967b, Clement 1917, Dissescu 1963, Keremidchiev 1972, Maksimović 1954, Patočka and Čapek 1971, Semevsky 1973, Szalay-Marzsó 1957, Vasić 1958, Vasić and Janović 1958). In dense Glenville populations, the direct contribution of variation in fecundity to overall variation in egg density was rather small, but positive correlations between fecundity and the survival rates of large larvae and pupae served to magnify this contribution considerably (Campbell 1967b).

Edel'man (1963) showed, that stages in gypsy moth development are related to the biochemical composition of the leaves, and Werner (1978) found that repeated defoliations by the spear-marked black moth, *Rheumaptera hastata* (L.), result in trees that produce "An abundance of nutrient depleted food . . .

Figure 4-12.—*Relationships among gypsy moth population density, site soil-moisture conditions, and larval disease incidence (Glenville, N.Y., 1958-61)* (*Campbell 1963*b).

[hence] ... larval starvation and population decline." Defoliation also reduces water losses and water stress (Stephens et al. 1972). Little direct evidence appears to have been accumulated on the gypsy moth regarding a possible feedback between current defoliation and a subsequent decrease in foliage nutrients. However, several authors have noted that equally dense gypsy moth populations cause more defoliation early in an outbreak than later on (Campbell and Standaert 1974, Dissescu 1963). Cambini (1975) concluded that defoliation of cork oak results in delayed bud burst in the following year.

Most of the adult insects tend to be males in the year of population decline (Campbell 1963a, Campbell 1967b, Keremidchiev 1972, Vasić 1950, Vasić and Janković 1958).

Changes in Stand Composition

Heavy, repeated defoliation by the gypsy moth can result in dramatic forest responses, at least in North American forests. These responses can reduce the capacity of the forest to sustain an outbreak.

Differential loss rates among favored-food and less-favored trees in the composite Melrose Highlands forest tended to result in residual stands less susceptible to defoliation than those present originally
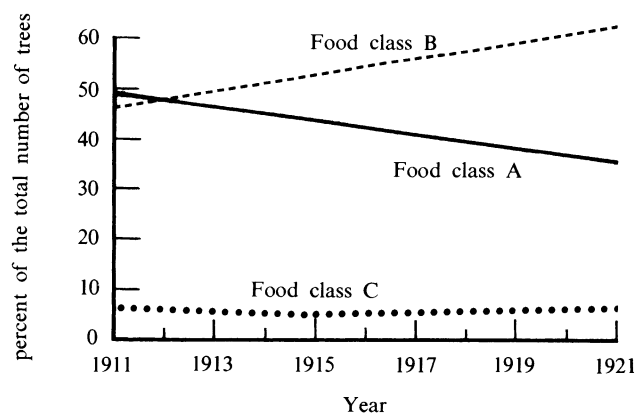
Table 4-10.—*Status of gypsy moths that were placed in the undetermined category: Old Lyme vs. Eastford*

Status	Old Lyme		Eastford	
	Number	Percent of total	Number	Percent of total
Virus[1]	35	15.5	16	8.4
Micro-organism[2]	51	22.6	86	45.3
Wound	65	28.8	32	16.8
Virus and micro-organism	22	9.7	10	5.3
Virus and wound	19	8.4	5	2.6
Micro-organism and wound	22	9.7	37	19.5
Virus and micro-organism and wound	12	5.3	4	2.1
Total	226	100	190	100

[1]Fewer than 10 polyhedra per field.
[2]Includes both aerobic bacteria that did not prove to be pathogenic and fungal mycelia or spores.
Source: Campbell and Podgwaite 1971.

(fig. 4-13). Recent stand composition changes in New Jersey and Pennsylvania have led others to conclude that subsequent outbreaks by this pest may not be as frequent or damaging as they tend to be after initial invasion (Houston and Valentine 1977, Kegg 1971, 1973, Nichols 1961). Capinera and Barbosa (1977) concluded that forest composition may directly affect population quality, as well as numerical levels.

In Eurasian forests, stand responses to defoliation appear to be relatively mild. Heavy, repeated defoliation seems fairly common (Baeta Neves 1947, Rafes 1970, Romanyk 1965, Turchinskaya 1963), and increment loss from defoliation is often significant (Fratzian 1973a, Magnoler and Cambini 1968, Mirković and Miscević 1960, Vorontsov and Mozolevskaja 1972). Different species have different susceptibilities to defoliation (Fratzian 1973b), but tree mortality is usually surprisingly low (Rafes 1970, Romanyk 1965, Turchinskaya 1963).

One possible explanation for the apparently more benign gypsy moth/forest relationship in Eurasia, as opposed to that of North America, is described in the section on long-term gypsy moth/forest relationships.

Figure 4-13.—*Percentage of total live trees on the Melrose Highlands plots that were favored food (class A), eaten but not favored (class B), and not usually eaten (class C), for each year between 1911 and 1921 (Campbell and Sloan 1977a).*

Factors That Distort the Sex Ratio

Some mortality factors kill subadult male and female gypsy moths at different rates. In extreme cases, these factors can cause phenomenal departures from the expected 50:50 sex ratio. Since these factors include some of the principal mortality processes in this life system, the percentage of females among adults at the end of the generation is sometimes closely related to trends in egg density from one generation to the next (Campbell 1963c).

Adult sex ratios in Glenville ranged from 83 percent females to only 2 percent females, but sex ratios were 50:50 among embryos and newly hatched larvae. The sex ratio at the start of the fourth instar was consistently about 65 females to 35 males (Campbell 1963c, Campbell 1967a).

Disease and probably other overpopulation phenomena (desiccation, starvation) are strongly selective against female larvae (fig. 4–14). This selection occurs mainly because these phenomena usually peak as the insects are pupating. Because male insects tend to pupate before females, the females are more vulnerable. In Glenville, only 10 percent of the pupae were females after one epizootic.
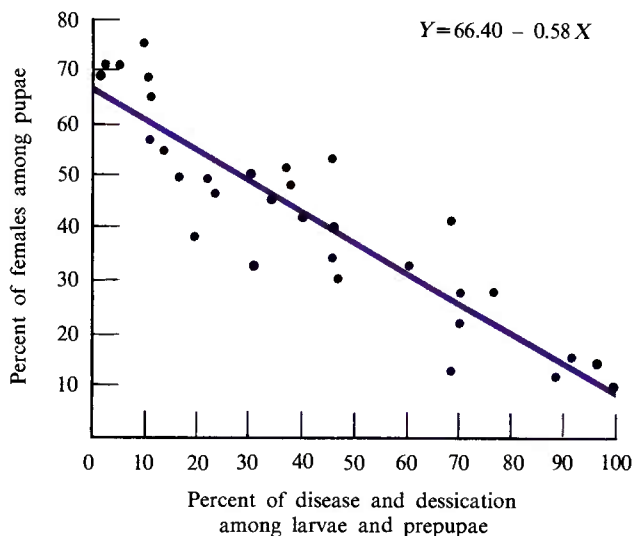

$Y = 66.40 - 0.58 X$

Figure 4–14—*Percentage of females among pupae as related to disease and desiccation among fourth-through sixth-instar larvae and prepupae (Glenville data) (Campbell 1963b).*

As indicated earlier, white-footed mice are more likely to prey on female than male pupae. In Eastford, this caused a shift from about 50 percent females among pupae to only about 25 percent females among adults (Campbell 1976, Campbell and Sloan 1976, 1977b).

Ichneumonids usually kill more male pupae than females, except where pupal size is reduced by excessive density. Apparently, these ichneumonids tend to concentrate on host pupae of a given size (Campbell 1963c).

Finally, at least one tachinid, *Blepharipa pratensis* (Meigen), tends to kill more female pupae than males. These parasites deposit their microeggs on foliage, where female gypsy moth larvae are probably more likely than males to ingest them simply because females consume more foliage (Burgess and Crossman 1929).

Long-Term Gypsy Moth/Forest Relationships

Over time, forest succession will inevitably alter forest susceptibility to the gypsy moth. Within each host species, reductions may also be evolving in susceptibility to defoliation and damage. Both patterns of forest succession and possible evolving host/pest interactions are discussed further.

Forest Succession

Transient agriculture, heavy cutting, and wildfire have resulted in a vast area of second-growth, mostly even-aged stands in the hardwood forest of Eastern North America. Most of these stands are now from 50 to 90 years old (Marquis 1977). Changes are rapid in these young stands.

Various oak species are now dominant across much of this young forest, and oaks may even increase temporarily, because of natural mortality among pioneer species (Collins 1962, Olson 1965). Except under exceptionally dry conditions, however, succession in such stands generally leads toward a species composition characteristic of mesic sites (Bess et al. 1947, Carvell and Tryon 1961, Clark and Watt 1971,

Niering 1953, Spurr 1956, Trimble 1973). Within such stands, defoliation-induced changes have usually tended to accelerate succession (Bess et al. 1947, Campbell 1978b, Campbell and Sloan 1977a, Clement and Nisbet 1972, Stephens 1976).

In contrast to the above, the gypsy moth may retard succession on dry sites. Oak stands on such sites may represent climax or at least relatively stable subclimax communities (Little and Moore 1949, Niering 1953, Oosting 1942).

Over time, the dynamics of North American gypsy moth populations are bound to be profoundly influenced by patterns of forest succession. Conversely, defoliation can also influence these successional patterns (Best et al. 1947, Campbell 1978b, Clement and Nisbet 1972).

Evolving Host/Pest Interactions

North American investigators have long recognized that susceptibility to gypsy moth defoliation varies among individuals within a species (Minott and Guild 1925). Differential tree mortality within each host species is a major consequence of this differential defoliation (table 4-11). In the Melrose Highlands forest, some trees within each species were consistently more heavily defoliated and, subsequently, more likely to die.

From the above results, Campbell and Sloan (1977a) inferred that a process similar to Pimentel's

Table 4-11.—*Mean tree-defoliation levels and subsequent mean percent tree mortality of three cohorts[1] for successive years following an initial defoliation of at least 20 percent by the gypsy moth*

	Defoliation and mortality subsequent to year of cohort establishment					
	Mean percent defoliation			Mean percent tree mortality		
Species	High range	Medium range	Low range	High range	Medium range	Low range
White oak	33.9	27.7	25.5	53.3	36.7	34.7
Red oak	20.1	17.6	16.3	24.3	14.6	10.3
Black oak	25.6	23.3	22.5	54.5	48.4	42.1
Scarlet oak	32.9	31.8	27.2	43.9	38.1	23.7
Gray birch	38.1	35.2	36.7	64.8	61.1	59.3
Paper birch	26.0	25.3	22.4	41.1	35.2	32.8
Red maple	22.3	16.6	14.0	33.7	25.8	22.6
White pine	10.4	5.9	5.0	37.3	16.8	14.9
Chestnut	9.2	8.5	8.0	77.1	75.1	75.0
Beech	14.0	9.6	10.0	6.2	0	4.2

[1] Each tree's cohort was established the first year in which defoliation of the stand reached 20 percent. Trees in the high range were defoliated more than average for the species; in the medium range, average defoliation; in the low range, less than average.

Source: Campbell and Sloan 1977a.

(1961, 1969) genetic feedback mechanism was operating in this life system. This process would tend to favor trees that suffered less than average defoliation and could be expected to result in a more defoliation-resistant forest than its predecessor (fig. 4-15). Largely through this process, forest stand responses to defoliation by the gypsy moth may eventually become similar to the relatively benign situation on other continents.

Historical Resumé

Around the world, most gypsy moth infested areas appear to support a potentially numerically bimodal population system. A predator complex that is probably dominated by vertebrates (birds and possibly small mammals) may be able to maintain sparse populations at innocuous levels indefinitely. Conversely, intrapopulation dispersal phenomena,
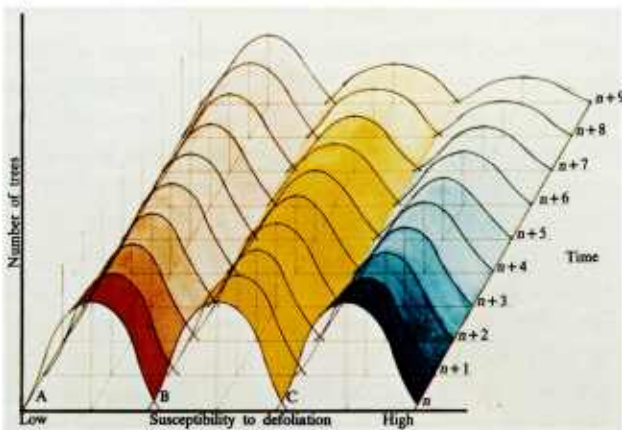

Figure 4-15.—*Change through time in apparent intraspecific susceptibility to defoliation (adapted from Campbell and Sloan 1977a).*

together with a series of possible adaptations by this insect to a numerically bimodal way of life, are also capable of maintaining an areawide outbreak for at least a decade.

In North America, spectacular outbreaks are relatively common near the advancing front of the generally infested area. However, several processes tend to modify this situation in areas that have been infested for several decades or more. These processes include responses by the gypsy moth and some of its introduced enemies to a new environment and responses by both native natural enemies and the forest stand to the gypsy moth.

Natural Regulating Factors

Introduction
William E. Wallner

To elucidate further the population dynamics of gypsy moth, a series of 0.04-ha plots was established in seven geographic regions. The intent was to develop techniques for estimating the population over time and to measure causes of mortality and distribution of life stages. Additionally, tree condition (starch and pathological state), small mammal predators, and parasites were to be documented. Initially, refinement of techniques was to be done in one location and then applied to the remaining six plots. However, because of the urgency of the gypsy moth problem, the decision was made to activate the complete system immediately. It was then discovered that the original estimates of manpower required and efficiency of techniques were not realistic within the existing economic confines.

Support through the expanded gypsy moth program was not forthcoming until after populations within the intensive plot system had declined. Thus, only partial information (egg-mass density and tree condition and mortality) was obtained. However, there have been a number of studies supported through the program that add substantially to a more thorough understanding of gypsy moth. Following are reports of this rather diverse research effort.

Parasites

General Considerations
Richard C. Reardon

The stabilizing influence of natural enemy populations in ecosystems has been reviewed by numerous authors: Simmonds 1956, Balch 1960, Turnbull and Chant 1961, DeBach 1964, 1972, Hagen et al. 1971, Munroe 1971, Hagen and Franz 1973, Coppel and Mertins 1977, and Ridgway and Vinson 1977. Pschorn-Walcher (1977) further characterized this stabilizing influence by contrasting the procedures and underlying philosophies of biological control in the forest ecosystem with those in agroecosystems and suggested that because of permanence and continuity of forest ecosystems, detailed preintroduction studies into the structure of parasite/predator complexes are both warranted and profitable. Unfortunately, there are no reliable criteria for determining a priori which components of the natural enemy complex will be effective if they establish.

The relative "degree of success" of biological control operations has ranged from complete failure to complete success for the gypsy moth, depending upon those criteria considered important. For example, DeBach (1964) listed four criteria: The proportion of the toal range of the pest in which control is achieved, the degree to which the duration or severity of outbreaks is reduced or the duration of the intervals between outbreaks is increased, whether supplementary control methods must be used, and whether a pest is of great or slight economic importance. Using these criteria, he rated the biological control program against the gypsy moth as a "partial success." In a more recent review, Reynolds (1976) evaluated the success of the Federal-State gypsy moth parasite program using five criteria; she concluded that the program was a "success" within the framework of time, money, and other resources allocated for its accomplishment. In general, the chances of success of biological control are less when the introduced pest (in this case gypsy moth) is also a pest in its native habitat, and when the forest to which

it has been introduced is highly favorable to its increase or vulnerable to its attack (Balch 1960).

The role of parasites as an *individual* regulating mechanism of forest insects has been discussed by numerous authors: Clausen 1956, Dowden 1962, DeBach 1964, 1974, Swan 1964, Turnock and Muldrew 1971, Morris 1959, Nielson and Morris 1964, and Coppel and Mertins 1977. In the most recent review by Coppel and Mertins (1977), several attributes of parasites were listed that should be considered for the use of parasites in biological control: Ecological compatibility, temporal synchronization, density responsiveness, reproductive potential, searching capacity, and dispersal capacity. In general, the most desirable parasite will respond positively to density, although such responses are of two types—functional and numerical. Positive responses of either type are beneficial to pest suppression, although a strong functional response alone is seldom able to regulate pest densities over many generations. A rapid and strong numerical response characteristic is the most important attribute of a successful parasite.

Foreign Studies
Richard C. Reardon

At least 150 species of parasites have been recorded from the gypsy moth throughout its Old World distribution. The species composition of parasites of the gypsy moth varies throughout Eurasia (Howard and Fiske 1911), although because of taxonomic difficulties (misidentification and synonyms), the number, relative importance as mortality factors, and distribution of the various species are difficult to evaluate among countries.

The species considered important throughout Eurasia are the egg parasites (*Ooencyrtus kuvanae* and *Anastatus disparis*), small larval parasites (*Apanteles* spp., *Meteorus* spp., and *Phobocampe disparis*), large larval parasites (*Blepharipa pratensis, Parasetigena silvestris, Exorista* spp., *Palexorista* spp., *Carcelia* spp., and *Compsilura concinnata*), and pupal parasite (*Brachymeria intermedia*). Total percent parasitism by complexes of these species

varies tremendously (less than 10 percent to 100 percent) in relation to geographic location, host density, and phase of cycle for alternate and/or overwintering hosts. For example, in the town of Kiev, Russia, a dense population of gypsy moths was apparently suppressed in 1908 through the activity of the parasites and only a few isolated colonies survived, whereas in the town of Bendery, Russia, in 1909, the damage to the forests was enormous and parasite control seemed to be most inefficient (Howard and Fiske 1911). More recently, Sisojević (1970) in Yugoslavia stated that oligophagous tachinids reached their maximum percentages (50–60 percent) on an intermediate host density, whereas the polyphagous species attained their highest percentages (10–20 percent) at relatively low gypsy moth density associated with a high density of alternate hosts.

North American Studies
1900–70
Richard C. Reardon

A total of nine exotic parasite species of the gypsy moth from Eurasia were established in North America from 1900 to 1960. (This does not include *Monodontomerus aereus* Walker, which is considered primarily a hyperparasite.) These nine species included most of the major parasites of the gypsy moth found in Eurasia: Egg parasites (*O. kuvanae* and *A. disparis*); larval parasites (*A. melanoscelus, P. disparis, B. pratensis, P. silvestris, E. larvarum,* and *C. concinnata*); and a pupal parasite (*B. intermedia*). *Apanteles porthetriae, A. liparidis, Exorista* spp., *Palexorista* spp., and *Carcelia* spp. were major species introduced in North America that were considered important elsewhere but that failed to become established, probably because of the lack of suitable alternate and/or overwintering hosts or poor colonization. However, it is not possible, because of differing biotic and abiotic environmental factors, to predict accurately the performance of exotic parasite species in North America on the basis of observations made overseas—some will exceed expectations, while some species will be less effective.

The early field work (1908 to 1910) conducted by Howard and Fiske (1911) showed that a maximum sixfold increase prevailed whenever the gypsy moth was in periods of innocuousness; an aggregate parasitism of 85 percent would be sufficient and much less than 75 percent would not be effective to maintain such a host density. During the early 1900's, an attempt was made to find in the literature such a degree of parasitism in Eurasia, but the scarcity of published information led to the conclusion that in few instances was existing parasitism sufficient to answer the requirements (at least 75 percent parasitism) in North America. In others it is obviously insufficient; in most the results of the study of imported material were not sufficiently reliable to support either contention. Also, at that time, it was generally accepted that the maximum rate of increase exhibited by the gypsy moth during progradation and culmination could only be met by an unreasonable percent of parasitism, thereby making the proposition of introducing parasites to prevent outbreaks unjustifiable.

By the end of the second importation effort, 1922–33, approximately 35 species of exotic parasites had been released in New England, although evaluation of their effectiveness was complicated because of chemical insecticide eradication and control attempts (that is, some parasite species might have been eliminated directly or indirectly through changes in gypsy moth density and/or changes in alternate and overwintering host populations).

Prior to 1960, several intensive studies (Melrose Highlands 1911–31, Bess 1961) of naturally occurring gypsy moth populations in North America were conducted in an attempt to "understand" the causes of periodic fluctuations of gypsy moth populations. These studies documented parasites as a contributing mortality factor but were not identified as the "major" mortality factor. Also during this time, generalizations concerning the role of natural enemies as mortality factors of the gypsy moth were numerous: Clausen (1956) stated "... gypsy moth outbreaks were reduced in range and severity comparable to those occurring in Europe due to natural enemies";

Friend (1945) said "... natural enemies were well established on the gypsy moth and were affecting its abundance"; and Turner (1963) stated "... it is assumed that parasites and predators are responsible for the low level of the pest between outbreaks and may also be responsible for increasing the interval between outbreaks." The relationship between rate of apparent parasitism and importance as a regulating factor is difficult to determine as discussed by Coppel and Mertins (1977) who said that the most numerous natural enemy of a pest is not always the most important one in regulating its density. A common parasite may simply be a part of an overall complex of mortality factors that cause a large and consistent reduction in host numbers. This leaves a small, low-density residual host population responsible for maintaining reproduction of the species—hence the desirability for a parasite to utilize this low-density population.

1970 to Present
Richard C. Reardon

Previous studies concerning the role of parasites as a mortality factor of the gypsy moth were conducted, for the most part, in the "old infested" areas and there was some doubt that those conclusions would apply to "newly infested" areas or could be duplicated in the early infested areas. Therefore, several studies were initiated in the early 1970's by various Federal and State agencies and universities in an effort to document the role of parasites in both newly and early infested areas. The results of these studies are summarized by State, except for the studies conducted by Hedlund and Reardon, which include two or more States. For additional information concerning specific collecting and rearing techniques and plot locations within individual States, see the following publications: New York, Tigner 1974, Reardon 1976; Massachusetts, Barbosa et al. 1975, Reardon 1976; New Jersey, Reardon 1976, Hedlund and Angalet 1978, Metterhouse 1978; and Pennsylvania, Hedlund and Angalet 1978, Ticehurst et al. 1978.

General Survey of Western Massachusetts
Pedro Barbosa

A survey of parasites emerging from gypsy moths collected from relatively high- and low-density sites was conducted in western Massachusetts (Barbosa et al. 1975). Of all the species recovered, *Compsilura concinnata* was the most abundant (about 27 percent parasitism), followed by *Blepharipa pratensis* and *Apanteles melanoscelus. Phobocampe disparis, Parasetigena silvestris,* and *Exorista* sp. were also recovered. The subtle influence of nucleopolyhedrosis virus, particularly at high densities, on the degree of recovery of parasites from hosts is unknown. Nevertheless, it appears clear that percent parasitism was greatest at the low gypsy moth density site. Similarly, parasitism by *Blepharipa pratensis* was more consistent and higher in the low-density site (table 4–12). This difference may have been affected by virus infection of larvae.

Parasitism by *C. concinnata* was highest in the lower canopy of the low density site. This differential may have resulted from microhabitat preferences of the fly or microdistribution differences of its host.

Parasite/Host Succession System in Pennsylvania
Mark Ticehurst

The objective of the parasite/host succession systems was to determine the species composition and relative importance of gypsy moth parasites along the "leading edge" of the infestation. This project (Ticehurst et al. 1978) was initiated in 1974 when 34 plots of 0.5 ha each were established in building first-cycle gypsy moth infestations in Schuylkill, Lebanon, Centre, and Union Counties along the "leading edge" of the infestation. Gypsy moth EM density ($\bar{x}$) peaked in 1975 and declined each successive year through 1979. Heavy defoliation occurred in 1974 and 1975, while light or no defoliation was observed in subsequent years.

More than 19,955 parasites were recovered from 110,797 larvae and pupae; the corresponding rates of parasitism are shown in table 4–13. These results represent the mean of the highest parasitism rate

Table 4–12.—*Comparison of parasitism of larval gypsy moths by* Blepharipa pratensis *(Meigen) in a low-density egg-mass site (Cadwell Forest) and a high-density egg-mass site (Town Forest)*

Week of collection	Percent parasitism	
	High-density site	Low-density site
2	0	0 .3
3	0	1.2
4	0	.3
5	.7	1.8
6	9.9	8.1
7	3.4	32.8
8	0	23.5

averaged from all plots each year. *B. pratensis* achieved the greatest rate of parasitism in 1974, *B. intermedia* in 1975, and *P. silvestris* in 1976–1978.

Parasites of Small Larvae

Apanteles melanoscelus parasitized 0.6, 0.7, 9.9, and 7.2 percent of the small larvae from 1974 to 1977, respectively. The highest rate observed was 29.0 percent ($n=101$) in 1978. This braconid appears to be the most influential following the collapse of the host infestation as seen in 1976, 1977, and 1978.

Phobocampe disparis was not recovered in 1974; however, it parasitized 0.1, 4.0, and 6.5 percent of the small larvae from 1975 to 1977, respectively. A sample of 102 larvae collected in 1977 produced 30 *P. disparis,* indicating 30.4 percent parasitism. Parasitism rates were highest in sparse host populations following a gypsy moth collapse.

Parasites of Large Larvae

Compsilura concinnata was recovered from 5.9 percent of the larvae in 1974, 0.9 percent in 1975, 2.1 percent in 1976, and 2.3 percent in 1977. The highest rate observed was 53.6 percent ($n=262$) from a collection in 1974. Parasitism appeared to be greatest in sparse building gypsy moth populations preceding a collapse. However, because it is broadly polyphagous, its influence is probably directly related to the presence of alternate hosts.

Table 4-13.—*Percent parasitism of gypsy moth in parasite/host succession plots, 1974-78*

Year	Egg masses per hectare ($\bar{x}$)	Percent parasitism					
		A. melanoscelus	P. disparis	C. concinnata	P. silvestris	B. pratensis	B. intermedia
1974	4,819	0.6	0.0	5.9	0.4	21.4	9.8
1975	7,051	.7	.1	.9	2.3	8.4	29.8
1976	1,141	9.9	4.0	2.1	22.5	12.7	.5
1977	220	7.2	6.5	2.3	49.7	24.4	.6
1978	233	9.7	12.4	4.6	61.9	4.8	0

Parasetigena silvestris was first observed in Pennsylvania in 1970 despite its establishment in New England in 1927. This parasite increased dramatically, killing 0.4, 2.3, 22.5, 49.7, and 61.9 percent of the large larvae from 1974 to 1978, respectively. The highest rate, 83.0 percent (n=100) was observed in 1978. *P. silvestris* was a major mortality factor in sparse gypsy moth populations in 1977 and 1978, the second and third years following the collapse of the host population.

Parasitism by *P. silvestris* was much greater in Pennsylvania than in other Northeastern States and was equal to or greater than maximum parasitism observed in Yugoslavia over a 16-year period (Sisojević 1970). The ecological conditions in central Pennsylvania, the southwestern extent of the defoliating infestation, may be more optimal for *P. silvestris* than areas to the northeast.

Blepharipa pratensis was recovered from 21.4 percent of the large larvae and pupae collected in 1974, 8.4 percent in 1975, 12.7 percent in 1976, and 24.4 percent in 1977. The greatest rate, 53.8 percent (n=200), was observed in 1974. Parasitism by *B. pratensis* appears to be inversely related to defoliation and host density. Lack of foliage during periods of heavy defoliation would prevent *B. pratensis* from ovipositing on foliage and thus would prevent gypsy moth larvae from ingesting eggs and becoming parasitized (Sisojević 1970).

Parasites of Pupae

Brachymeria intermedia parasitized 9.8 percent of the pupae collected in 1974, 29.8 percent in 1975, 0.5

percent in 1976, and 0.6 percent in 1977. The highest rate observed was 67.2 percent (n=197) in 1975, from a dense host population that resulted in heavy defoliation. Parasitism appears to be directly related to host density and/or defoliation. Drastic changes in parasitism by *B. intermedia* (that is, 29.8 percent in 1975 to 0.5 percent in 1976 in same sites) may indicate massive dispersal of the parasite population into or out of more or less desirable host populations.

These results indicate that parasitism by most gypsy moth parasites is related to host density and the phase of the host cycle. Parasitism by *A. melanoscelus*, *P. disparis*, *P. silvestris*, and *B. pratensis* was greatest at relatively low host density in the first through third postculmination years following the collapse of the host population. However, parasitism by the pupal parasite, *B. intermedia*, was greatest during the culminating phase of the host cycle with peak host density and heavy defoliation.

These data when compared to those reported in New England, show that rates of parasitism in Pennsylvania are generally different than those reported elsewhere. For example, Barbosa et al. (1975), Tigner (1974), and Reardon (1976) indicated that parasitism by *P. silvestris* was minor, rarely exceeded 3 percent, in Massachusetts, New York, and New Jersey, respectively. The results of this study show that *P. silvestris* killed 61.9 percent of the large larvae in Pennsylvania, was a major mortality factor, and appeared to be largely responsible for massive stabilization throughout Central Pennsylvania (study area) in 1977-1979.

In summary, the parasite complex in Pennsylvania appears to be relatively ineffective (low rates of para-

Table 4-14.—*Gypsy moth parasites recovered in New York State*

| | Host stages affected | |
Parasite	Enter	Exit
Anastatus disparis Ruschka (Hymenoptera: Eupelmidae)	Eggs	Eggs
Ooencyrtus kuvanae (How.) (Hymenoptera: Encyrtidae)	Eggs	Eggs
Apanteles melanoscelus (Ratz.) (Hymenoptera: Braconidae)	Early, middle instars	Middle instars
Phobocampe disparis (Vier.) (Hymenoptera: Ichneumonidae)	Early instars	Middle instars
Compsilura concinnata (Meigen) (Diptera: Tachinidae)	Early, middle, late instars	Middle, late instars; pupae
Parasetigena silvestris (R.D.) (Diptera: Tachinidae)	Middle, late instars	Late instars, pupae
Blepharipa pratensis (Meigen) (Diptera: Tachinidae)	Middle, late instars	Late instars, pupae
Sarcophaga aldrichi Parker[1] (Diptera: Sarcophagidae)	Pupae	Pupae
Brachymeria intermedia (Nees) (Hymenoptera: Chalcididae)	Pupae	Pupae
Theronia atalantae (Poda) (Hymenoptera: Ichneumonidae)	Pupae	Pupae
Coccygomimus pedalis (Cress.) (Hymenoptera: Ichneumonidae)	Pupae	Pupae

[1]Relationships with host not completely known.

sitism) in preventing building host populations along the leading edge from reaching outbreak levels. Parasitism by *B. intermedia* during the culmination phase together with nucleopolyhedrosis virus and host stress are responsible for the collapse of the host population. Parasitism (60–70 percent) of the large larvae in the postculmination phase, primarily by *P. silvestris*, appears to be largely responsible for massive stabilization in 1977–1979.

New York Survey
Timothy Tigner

A cooperative survey was conducted in 1972 and 1973 by the Applied Forestry Research Institute, State College of Environmental Science and Forestry, and the New York State Department of Environmental Conservation (DEC) to determine the presence, distribution, and relative abundance of insects parasitizing the gypsy moth in New York State. Subsequent annual surveys by DEC have contributed supplemental records of larval and pupal parasitism in 25 counties (DEC 1976, Birmingham 1978).

Parasite species recovered from the gypsy moth in New York State are listed in table 4–14. All of these occurred in every county (25) surveyed. Apparent rates of parasitism were found to vary considerably among species, times and sites, and according to sampling method (Tigner 1974, Tigner et al. 1974). During the initial survey, *Blepharipa pratensis* was recovered at a much higher rate than other parasites in most areas, but *Parasetigena silvestris* has been more commonly obtained during the past 3 years. The greatest fluctuations in apparent parasitism have been recorded for *Compsilura concinnata*, presumably because of its dependence upon alternate hosts. The remaining species seldom emerged from more than 1 or 2 percent of field-collected host specimens.

The real contribution of parasites to gypsy moth population dynamics can be known only after a change in parasitism is demonstrated to cause a predictable change in generation survival of the host. This has not yet been done for any gypsy moth parasite in the United States. Any reference, therefore, to parasite "importance" or "effectiveness" is

Table 4-15.—*Peak parasitism totals by all parasites of gypsy moth larvae and pupae, based on weekly samples during each year of study*

Year	Location				
	Tuckahoe	Hawk Mt.	Hickory Run	Bald Eagle I	Bald Eagle II
1975	42	76	67	[1]49	54
1976	34	62	58	30	30
1977	60	50	41	[2]—	[1]68
Mean	45	63	55	40	51

[1]Gypsy moth populations collapsed here (populations in all other locations were relatively stable).
[2]Populations of gypsy moth larvae/pupae were too low for adequate sample size.

misleading. Gypsy moth parasites in New York were evaluated only on an empirical basis (Tigner 1974).

It is apparent that parasites have not prevented the gypsy moth from remaining a pest. The degree to which parasites influence the frequency or duration of host population outbreaks has yet to be elucidated. It is unlikely, in any case, that established species can be manipulated economically and predictably except under extraordinary circumstances.

New Jersey Permanent Gypsy Moth Plot System, 1970–77
William W. Metterhouse

The Permanent Study Plot System was established in New Jersey in 1970 to study the gypsy moth populations within a woodland environment, with special attention devoted to the evaluation of biological control factors affecting the gypsy moth. The system, as originally established, was composed of 20 permanent plots, each approximately 1.6 ha in size, in areas of varying degree of gypsy moth population conditions. Since 1970, most of the plots have advanced through the various progressive population cycles, and certain trends in gypsy moth population have been observed in the relationship of various parasite species.

Years of survey results indicate the establishment of seven species of parasites that were released and established in the New England States during the years 1905 to 1933. In addition, native parasites (three pupal and five larval) and predators (four predaceous beetles) have been found to attack the gypsy moth in varying degrees.

As a result of survey efforts, which have closely monitored the gypsy moth as it advances through

New Jersey, the trends in parasitism as related to the different gypsy moth population levels have been recorded. In the preoutbreak stage, the tachinid larval parasite, *Compsilura concinnata*, is the first parasite to be observed. This parasite was established in New Jersey prior to the introduction of the gypsy moth, having been recovered on alfalfa caterpillar, imported cabbage worm, and other native hosts. In the outbreak or culmination years, the tachinid larval parasite, *Blepharipa pratensis*, the braconid larval parasite, *Apanteles melanoscelus*, and the chalcidid pupal parasite, *Brachymeria intermedia*, attain the highest rate of parasitism. In the postculmination years, the tachinid larval parasites, *Parasetigena silvestris* and *Compsilura concinnata*, exhibit the highest percentage of parasitism and thus appear to be contributing importantly to the dampening or stabilizing of the gypsy moth population.

Other parasites acting less significantly in the stabilized areas are the tachinid parasite, *Blepharipa pratensis*, and the braconid parasite, *Apanteles melanoscelus*. The ichnemonid larval parasite, *Phobocampe disparis*, and the predaceous beetle, *Calosoma sycophanta*, are not widely established, although *P. disparis* is being recovered in more places each year. The egg parasite, *Ooencyrtus kuvanae*, expresses maximum benefit during the year of gypsy moth collapse and years of stability.

Only continued years of monitoring will provide more complete answers, but present results indicate, in stable areas, that parasites are host-density dependent and appear to be factors contributing to stability following the viral collapse of the gypsy moth population. Wide-scale stabilization has occurred in

New Jersey, and more recently, some of these areas are reexploding with damaging levels of gypsy moth populations. Furture investigations of these reoccurring populations should yield interesting facts about parasite/host relationships.

Observations on Parasites of the Gypsy Moth in Pennsylvania and New Jersey
Robert Hedlund

Field collections of gypsy moth eggs, larvae, and pupae were made during 1975–77 at five locations in New Jersey and Pennsylvania. The objectives of these collections were to determine which parasites of the gypsy moth were present; the percentage of the host population being parasitized; the differences, if any, that existed in the species; and the effectiveness of the parasites at different locations.

When possible, 100 larvae and/or pupae were collected from each of 13 collection sites (distributed among the five locations) each week beginning the second week after hatch. These were reared in the laboratory and observed for parasite emergence. Twenty-five egg masses were collected from each site during the winter months and dehaired in the laboratory, and the number of parasitized eggs were counted.

The maximum parasitism (during the 8–10 week collection period) observed at each location in each year is shown in table 4–15. Each number is the largest percentage parasitism (total of all species of parasites) detected during the season on a single sampling date. Parasitism generally was low in the early instars and increased as the larvae matured; however, because the gypsy moth population is constantly declining, it should be emhasized that 10 percent parasitism in the early larval stages may be killing more individuals than 50 percent parasitism of the pupae. In addition, the "peak total parasitism" used here will always be a conservative index because part of the parasitism that occurs earlier (and/or later) is not counted at the peak but does contribute to the total population mortality.

In most years and locations, observations show that nearly 30 percent of the gypsy moth egg population is destroyed by *Ooencyrtus kuvanae*. In addition, 30 to 76 percent of the gypsy moth pupae and large larvae were killed by a complex of parasites. Parasitism of early instars by *Apanteles melanoscelus* and *Compsilura concinnata* was low and decreased at most locations during the 3 years, while parasitism of large larvae by *Blepharipa pratensis* and *Parasetigena silvestris* was moderate to high and increased during the study. The pupal stage was seldom attacked by parasites (although many larval parasites emerge during this stage), except in most areas of heavy defoliation, when *Brachymeria intermedia* was a significant parasite.

In summary, these studies have shown that parasites are more consistent and effective than had previously been supposed, killing nearly 30 percent of gypsy moth eggs and at least an additional 40–60 percent of the larger larvae and/or pupae collected. No one species of parasite was superior in all years and locations, indicating that a complex of species is necessary for consistent mitigation of gypsy moth populations. Introduction of additional parasite species, especially those that attack small larvae, or pupae, would be desirable in order to increase the total mortality by parasites.

Forest Service Intensive Plot System in Massachusetts, New York, and New Jersey
Richard C. Reardon

The Intensive Plot System established by the Forest Service in 1972 (Campbell and Bean 1971) comprised six study areas of 12 ha each in three States: Two each in Massachusetts, New York, and New Jersey (fig. 4–16). Each study area was divided into six sites of 2 ha each. In 1972 and 1973, four techniques were used to collect host larvae and pupae for parasite recovery: General; stratified or 1/100-ha plot, 1972 and 1973, respectively; burlap-band; and burlap band/tree species (Reardon 1976).

In 1972, a total of 112,431 gypsy moth larvae and pupae was collected, and total parasitism varied between 4 and 17 percent in the different areas. Among all areas, three species of tachinids— *Blepharipa pratensis* (Meigen), *Compsilura concinnita* (Meigen) and *Parasetigena silvestris* (Robineau-

Desvoidy)—accounted for 75.8 percent of the parasites recovered. *B. pratensis* comprised 67.8 percent of the total; the braconid *A. melanoscelus* accounted for 15.8 percent.

Parasite incidence was higher for host collections from the tree bole than from the foliage (canopy) or litter, while parasite species composition was similar for the three strata. Also, there were more *B. pratensis* and *P. silvestris* per larva collected under burlap bands than by collections from ground, bole, and foliage without the use of artificial niches.

In 1973, a total of 53,379 gypsy moth larvae and pupae were collected, and total parasitism varied between 3 and 18 percent in the different areas. The recoveries of *A. melanoscelus* were down to approximately 8 percent of the total parasitism, whereas the combined parasitism of the 3 species of tachinids increased to 78.4 percent of the total measured parasitism. *B. pratensis* comprised approximately 33 percent of the tachinid total.
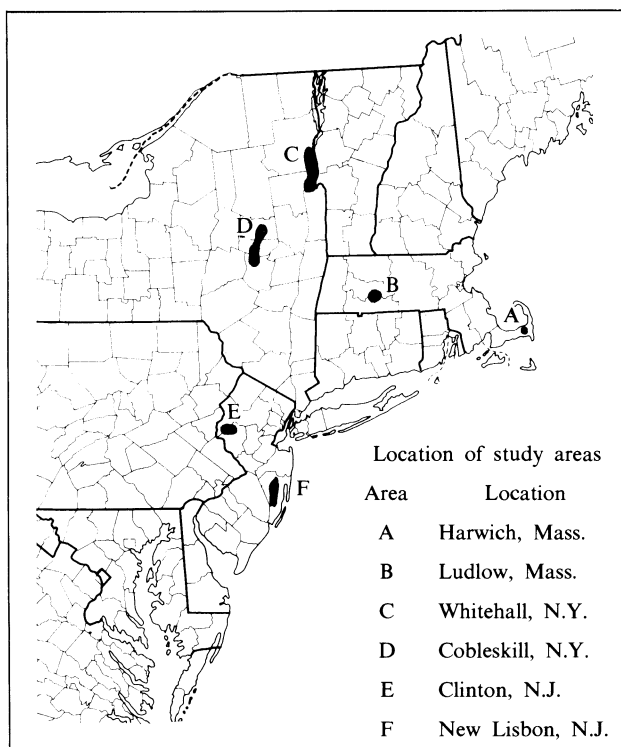

Location of study areas	
Area	Location
A	Harwich, Mass.
B	Ludlow, Mass.
C	Whitehall, N.Y.
D	Cobleskill, N.Y.
E	Clinton, N.J.
F	New Lisbon, N.J.

Figure 4-16.—*Location of intensive plot study areas in the Northeastern United States, 1972 and 1973.*

The chalcid *B. intermedia* (fig. 4-17) appears to be the only parasite whose numbers increased with percent defoliation, number of larvae and pupae, and number of egg masses per 0.4 ha. Numbers of the hyperparasite *B. compsilurae* were positively correlated with numbers of *B. intermedia* and percent defoliation, suggesting the attraction to open sunny areas of both hyperparasites and primary-parasites of the genus *Brachymeria* and indicating high host density.

In low host-population levels, the numbers of *P. silvestris*, *B. pratensis*, and *C. concinnata* were positively correlated with numbers of host immatures, while in areas of heavy defoliation, a negative correlation seemed to exist. This may indicate a critical level of defoliation, microclimatic change, or combination of related factors whereby the tachinid parasites demonstrate an avoidance mechanism. Sisojević (1975) found a similar situation in Yugoslavia for high-density larval populations of the gypsy moth whereby *B. pratensis* and *P. silvestris* were adversely affected. *B. pratensis* was affected by complete defoliation prior to its oviposition as this species oviposits a microtype egg on foliage (figs.4-18 and 4-19), whereas the abundance of gypsy moth larvae distributed ovipositing *P. silvestris* females (fig. 4-20). Also, *P. silvestris* females would not oviposit on starved and diseased host larvae; in fact, the reduced relative humidity in the completely defoliated stands forced *P. silvestris* to emigrate from such areas.

The general technique should be used for collecting host immatures to estimate parasite incidence, as this technique will provide data on parasite incidences and species composition without a treatment effect (that is, use of artificial niches). Whereas burlap-band and burlap-band/tree-species techniques should be used only in an attempt to maximize parasite recovery, because the incidences for *A. melanoscelus*, *P. silvestris*, and *B. pratensis* will be high.

The 1972 and 1973 average percentage of parasitism data (12 and 14 percent, respectively) as determined by four collection techniques indicates that parasites by themselves, as an individual mortality factor, did not remove a significant proportion of the host population and did not

function to limit the rate of increase of the gypsy moth in these areas. Nevertheless, parasites did remove a portion of the host population and in combination with other mortality factors would have an influence on the rate of increase of the host.

Parasite/Gypsy Moth Interactions: Summary and Suggested Areas of Future Research
Richard C. Reardon

Natural enemies are a stabilizing influence in ecosystems, although there is lack of agreement about the relative importance of parasites, predators, and pathogens as separate components of the natural enemy complex of the gypsy moth. Several of the parasite species of the gypsy moth considered important throughout Eurasia are established in North America: Egg parasites (*O. kuvanae* and *A. disparis*), small larval parasites (*A. melanoscelus* and *P. disparis*), large larval parasites (*B. pratensis, P. silvestris, E. larvarum,* and *C. concinnata*), and pupal parasite (*B. intermedia*). Nevertheless, some species considered important in Eurasia (*A. porthetriae, A. liparidis, Exorista* spp., *Palexorista* spp., and *Carcelia* spp.) have not been established in North America, probably because of the unavailability of alternate

Figure 4–17.—*Female adult* Brachymeria intermedia (*Ness*) *parasitizing a gypsy moth pupa.*

hosts. There are no reliable criteria for determining a priori which species will be effective if they establish, but this does not preclude the need to obtain preintroduction information on voltinism, host specificity, and diapause. The role of parasites as an individual mortality factor is difficult to interpret from both foreign and North American literature because several life stages of the parasites and host cannot be adequately sampled. The literature is rife with generalizations concerning parasites as being responsible for the low levels between outbreaks, reducing the severity of outbreaks, etc; however, supporting data have not been published either in the foreign or North American literature. In fact, the structure and interactions of the different species of the parasite complex in various geographical areas, forest ecotypes, and microhabitats are relatively unknown. For many species, data on alternate host requirements, dispersal, and impact of hyperparasites are lacking. One logical approach to gathering data on individual parasite species and complexes is a cooperative effort from the many organizations involved in the study of gypsy moth parasites. There is

an urgent need for the development and use of standardized techniques to sample host and parasite populations across many geographical areas. More intensive studies of those species established in North America as well as of exotic species are needed to understand their specific ecological requirements and interactions with other species. Coordination of parasite bioecology studies is essential in order to assess more accurately the role of parasites in regulating gypsy moth populations.

Additional studies are needed to elucidate the interaction of parasites and infectious diseases in populations of the gypsy moth. This interaction might be an important naturally occurring mortality factor and/or provide a mechanism whereby artificially contaminated parasites could transmit infectious diseases and create foci for the initiation of epizootics. Also, laboratory and field cage studies should be continued to evaluate the potential of introducing parasites from allied species to parasitize the gypsy moth.

Gypsy Moth Predators
Harvey R. Smith and Richard A. Lautenschlager

Introduction

The general role of predation in the population dynamics of the gypsy moth has been discussed by Campbell (Historical Review, in chapter 4), who concludes that predation can regulate certain sparse stable populations indefinitely. During the outbreak mode, as during the decline phase, predation has no significant impact, even though several bird species exhibit a numerical response during these periods of high prey abundance. In this section the interactive and differential components of predation are discussed as they relate to gypsy moth population dynamics.

Gypsy Moth Behavior and Predation

At sparse levels, the gypsy moth in North America exhibits an apparent defense behavior that evolved in its native European habitat—the migrating of larvae from the tree crown to the litter at the base of the host

Figure 4–18.—*Female adult* Blepharipa pratensis (*Meigen*).

tree. Campbell and Sloan (1976) suggested that this behavior evolved in response to natural enemies (birds and parasites) that were active in the canopy. In North America, this downward migration increases the insects' vulnerability to mammals and often results in high gypsy moth mortality.

Through natural selection, gypsy moth larvae developed defenses against predation. These defenses, such as warning coloration and stiff hairs, are particularly important from the fourth instar to pupation. Defense mechanisms such as these are common to many Lymantriidae larvae. Buckner (1966) pointed out that because of strong defense mechanisms there are few vertebrate predators of insects such as the forest tent caterpillar, *Malacosoma disstria* Hubner, and the gypsy moth.

Despite these defenses, which may have limited the number of gypsy moth predators in North America, many predators do exist. Some appear completely unaffected by these defense mechanisms; others have developed ways of coping with or circumventing them. It has been noticed that the shorttail shrew (*Blarina brevicauda*) often attacks gypsy moth larvae from the underside, removing organs and fluid, while leaving the larval skin and protective hairs intact. Yellow-billed cuckoos (*Coccyzus americanus*) that ingest the larvae whole periodically shed and regurgitate their stomach linings, which have become imbedded with the larval hairs (Wetmore 1965).

The migratory behavior of larvae raises some interesting questions about its effectiveness for survival of the species. In this country, birds still seem to be an important regulatory force (Campbell and Sloan 1977a) which may tend to reinforce the larval pattern of resting in litter at the base of a tree. But is this behavior really protective, since larvae in the litter are exposed to small mammals? Are the larvae surviving only to become prey in the pupal stage? In one study, Campbell et al. (1975a) showed that only those females that pupated above the litter (under bark flaps) had a reasonable survival probability.

Bess (1961) and Bess et al. (1947) indicated that in mesic forests survival is low when gypsy moths rest in the litter and innocuous populations predominate.

Campbell and Sloan (1976, 1977a) verified these observations and presented further evidence that vertebrates are essential in maintaining low populations. They estimated that vertebrates killed 70 percent of the pupae in a series of sparse, stable populations and that white-footed mice, *Peromyscus leucopus,* accounted for 40 pecent of those removed.

Gypsy moth larvae seeking a daytime resting location often select the dark tunnels of small mammals located just below the litter. In a sparse gypsy moth population in Mashpee, Mass., which showed no visible signs of defoliation, gypsy moth larvae apparently favored these small mammal tunnels as a resting location (Paszek 1977). An Animal and Plant Health Inspection Service (APHIS) field crew collected approximately 8,000 female sixth-instar larvae, prepupae, and pupae within a 52-ha area almost exclusively from small mammal tunnels located at the base of oak trees. Few pupae were found anywhere else. Small mammal predation was evident in these tunnels by the large number of pupal fragments found. This behavior of resting and pupating in small mammal tunnels has also been observed in a study area in Connecticut.

Perhaps 100 years has not been sufficient time for this behavior of migrating to a resting location to change; however, it appears that little selection exists against this behavior. Possibly the stimulus that causes larvae in sparse populations to descend to the litter and death for the majority may also insure a stability that would not be as likely if the insect remained in the tree. Such a possibility exists because in these populations significantly higher survival results when larvae rest above the litter (Campbell et al. 1975a, b). Higher survival could result in outbreak densities that would eventually be controlled by starvation or epizootics, both of which may reduce populations to very low levels far below that of predators (Craighead and Craighead 1969), hence a less stable situation.

Predation and Population Stability

In each life stage, the gypsy moth becomes prey to several animals, but until recently the role of predators

in gypsy moth population dynamics was not well documented. The predatory role of birds received most of the attention of early naturalists. Forbush and Fernald (1896), who based their findings on daytime observations, considered birds the primary predators of forest insects. They also mentioned amphibians, spiders, and insects as predators; however, mammals were ignored (except skunks were reported feeding on adult female moths). Investigators at that time seldom discussed nocturnal mammals or mentioned other vertebrates.

Hamilton and Cook (1940) emphasized that the beneficial role of small mammals in the economy of the forest had received little attention. Bess et al. (1947) were the first to suggest that small mammals (mice and shrews) were important predators of the gypsy moth. However, when Buckner (1966) described the role of vertebrates in biological control of forest insects, the situation still had not changed significantly. Although more species of birds have been identified as predators of the gypsy moth in this country, mammals may have more impact on gypsy moth populations.

An overview of the Eurasian literature earlier in this chapter indicates that birds are the only vertebrates recognized as important in regulating gypsy moth populations. However, the role of small mammals as predators of the gypsy moth in Eurasia is not well known. Possibly the level of protection afforded by defense mechanisms that the gypsy moth evolved in Europe may have been lessened in northeastern American forests because potential predators and habitats are different and the roles of birds and mammals in the United States uniquely complement each other.

Only Rotschild's study is known to deal with mammalian predators of the gypsy moth in Europe. He reported that the remains of gypsy moth larvae and pupae were found in the stomachs of *Apodemus flavicollis* and *A. sylvaticus* (Old World wood mice) and *Dyromys nitedula* (the tree dormouse) in the Soviet Union, and that the stomachs of these mammals are often filled exclusively with the remains of these insects. These European rodents, plus the smaller *Micromys* sp. (the harvest mouse), have characteristics that could make them important gypsy moth predators: All eat insects, and the forest dormouse and harvest mouse are skillful climbers.

Because there is little information in the literature, it is impossible to draw any firm conclusion about the role of European mammals in gypsy moth population dynamics. For example, one American scientist who

Figure 4-19.—Blepharipa pratensis (*Meigen*) *eggs on foliage.*

Figure 4-20.—*Female adult* Parasetigena silvestris (*R.-D*) *ovipositing on a gypsy moth larva.*

visited the Soviet Union in 1975 was told that *Apodemus* was not important in regulating gypsy moth populations (Lewis 1978).

In northeastern American forests, two common small mammals are important gypsy moth predators: The white-footed mouse (fig. 4–21) and the shorttail shrew (fig 4–22). Both readily eat gypsy moths, and the white-footed mouse commonly climbs trees to eat larvae, pupae and adults. Although European forests contain a variety of shrews, including several *Sorex* sp. (longtail shrews) and *Crocidura* sp. (the white-toothed shrew), there is no equivalent of the North American shorttail shrew.

Habitat differences also affect predator effectiveness. The long, dry summers typical of the south-central European forests where gypsy moths occur may cause intense vegetation competition for available moisture. This often results in forests with more grasses and fewer shrubs in the understory (Smith 1978). Such stands represent poor habitat for the small mammals that depend on dense shrubs for both food and cover and probably limit both the number and diversity of small mammals. Except on inherently xeric sites, such as ridgetops and sandy soils, forests in the Northeastern United States are usually quite moist and often have well-developed shrub and herb layers that offer both food and cover to small mammals and birds.

What is the relationship in North America between predation and gypsy moth population stability within the innocuous mode? This is difficult to answer because the specific factors that lead to an outbreak are not clearly understood. The hypothesis developed by Campbell and Sloan (1977a) regarding the determinants of numerical stability among the sparse populations that were studied stated, "...year-to-year numerical stability among these populations was determined largely by a combination of predaceous birds, which tended to concentrate on instar IV–VI larvae, and small mammals, especially *Peromyscus leucopus,* which tended to concentrate on the pupae." Their results further demonstrated the importance of predation. Predation, although it is a powerful natural force that operates simultaneously with other

physical and biotic forces such as climatic catastrophes, habitat limitations, food shortages, diseases, and parasites, has limitations. Predation does not exert constant pressure and as a suppressive force does not reduce populations to levels as low as those resulting from climatic catastrophes, starvation, and epizootics.

In their discussion of predation, Craighead and Craighead (1969) stated, "Although predation can be the limiting factor, we should perhaps have a truer concept of it if it were thought of not in terms of when and how it may assume this role, but rather as a regulatory force continually operating to lower prey increase in proportion to prey density and to do this before more drastic but less steadily functioning forces become effective."

Predation undoubtedly had a significant effect in maintaining the sparse-stable populations studied by Campbell and Sloan (1976, 1977a), Bess (1961), and Bess et al. (1947). However, it would be inaccurate to say that predators can *control* gypsy moth populations or prevent outbreaks; predation is simply one of many potential regulating factors. Although predators can reduce the threat of outbreaks when prey populations are in the innocuous mode, predation will not control outbreaks. At this writing, the precise role of predation in gypsy moth populations remains unclear, because of the complexity of the predator community and the many factors affecting predator potential.

Although gypsy moth population densities respond to predator pressure, periods of low predatory pressure would not necessarily lead to an outbreak. However, when low predatory pressure coincides with other population-releasing mechanisms, an outbreak would be more likely to occur. Campbell and Sloan (1977a) showed that when birds and small mammals were experimentally removed from an area, the gypsy moth population in that area could increase tenfold the following year. This increase clearly demonstrates the suppressive force of predation within sparse populations. The key to understanding the precise role of predation in sparse populations lies in the ability to identify alternative prey and foods, the

availability of which coincides with that of the gypsy moth and how abundance and predator preference for these foods affect the selection of gypsy moths.

Predation Potential of Birds and Mammals

Although birds and mammals have been recognized as important predators of the gypsy moth for many years, it is only recently that their precise roles within sparse gypsy moth populations have begun to be understood and appreciated.

Two unique attributes of birds and mammals allow them to achieve an economically important impact potential: They are warmblooded, and they have a highly developed learning ability. Because they are warmblooded they require a tremendous amount of food just to produce the energy necessary to maintain body temperature. Adult birds may eat an equivalent

of one-third their weight per day, and young birds often eat more than the equivalent of one-half their weight per day (Chapman 1968). One study of food consumption by birds and mammals in a 1,000-ha virgin forest in Czechoslovakia indicated that the bird population consumed food equaling about 25 percent of its weight daily; the mammal community consumed the equivalent of 20 percent (Turcek 1952).

Those mammals most useful as predators of forest insects—mice, shrews, voles—eat weight equivalents much greater than 20 percent of their own weight every 24 hours. Shrews are alleged to consume their own weight equivalent in food every day (Lowery 1974).

The other factor unique to birds and mammals is the degree to which certain functions of the brain are developed. Both birds and mammals learn to search

Figure 4-21.—*White-footed mouse,* Peromyscus leucopus.

out places where various foods are found, concentrate their foraging in those places, avoid insect defense mechanisms, and seek insects or parts of insects that are most palatable or desirable. For example, the white-footed mouse prefers the larger female pupae to the smaller male pupae and, after catching gypsy moth larvae, eats only a very small portion of the insect. Hoarding—a behavior associated with learning and well developed in mammals—has been demonstrated by shorttail shrews, which often gather pupae and carry them underground to be eaten later. An example of learning was observed on Cape Cod, Mass. (ODell 1977). Shortly after sunrise, blue jays (fig. 4–23) would begin to search systematically lower branches and tree trunks for adult male gypsy moths. Captured moths were swallowed whole. By midmorning, the jays changed their searching pattern: They searched the shrubs for adult moths on the underside of the foliage. Blue jays soon learned that these moths could be easily flushed by hitting the shrubs and became quite proficient at flying into the shrubs, flushing the resting moths, and capturing the insects in flight.

Both the activity patterns and the inquisitive and opportunistic behavior of birds and mammals make them particularly adapted for searching out and killing gypsy moths. Although diets will vary among forested areas, and impact potential can be lessened by the presence of other prey and food items, both birds and mammals can have a significant effect.

Perhaps one key to the effectiveness of bird and small mammal predation on sparse, stable gypsy moth populations lies in the behavior of the gypsy moth. The feeding response of a predator to a prey

Figure 4–22.—*Shorttail shrew,* Blarina brevicauda.

usually follows this pattern: At low prey densities, a few or no prey are attacked; at moderate prey densities, a steep rise in predation occurs; at high prey densities, predation levels off. This pattern would hold true for the gypsy moth and its predators if the insect did not migrate out of the canopy and aggregate in lower resting locations during the day. This prey behavior creates a unique situation; instead of the predator chasing the prey, the prey (gypsy moth) comes to the predator at dawn and dusk, when the insect is visible and attracting attention simply by moving, and when many vertebrate predators are at their peak activity.

By aggregating under loose bark or leaves in the litter, the insect is found in predictable resting sites that predators may have learned are rewarding when searched. The predators are thus able to exert much greater predatory pressure on sparse gypsy moth populations, which aggregate, than would be expected on insect prey (at low population densities) with a random resting distribution. Some population consequences of aggregation have been mentioned by Campbell et al. (1975a) and Campbell and Sloan (1977b).

Factors Affecting Predator Potential

The following variables affect predator potential:

• Prey abundance and availability (prey density, or the density at which predators recognize the prey as food and start to search for it).

• Abundance and availability of alternative foods (including other prey items).

• Density of predators.

Figure 4–23.—*Blue jay,* Cyanocitta cristata.

• Predator size.

• Predator period of residency and size of its feeding area.

• Predator willingness to eat the prey and the life stages preferred and eaten.

• Predator ability to capture and consume prey (prey defenses, conspicuousness, size, and palatability, and the same characteristics of the alternative food sources).

Several experiments have been conducted with caged mammals in order to better understand predator potential. The history of one experiment and two observations from natural situations illustrate the importance of several factors that affect predator potential.

In the experiment, six adult male white-footed mice were individually caged with 100 fifth-instar gypsy moth larvae and water. Two of these mice also received mouse chow (a dried laboratory food), and two others received mouse chow and sunflower seeds. Mice given no alternative food ate 98 percent of the larvae within 48 hours, mice with mouse chow ate 57 percent of the larvae, and mice with mouse chow and sunflower seeds ate 23 percent of the larvae. The effect these alternative foods had on the percentage of larvae eaten after 48 hours clearly demonstrates the selective feeding ability of the mice and appears to be similar to what happens in the wild where the number of prey eaten will depend on the availability of alternative foods.

A natural situation that illustrates the effects of alternative food, the defense mechanism of stiff hairs, and prey density on gypsy moth survival was recently observed in New Lisbon, N.J. (Garlo 1977). Nest boxes were placed on a 0.7-ha site as part of a small-mammal study. In early summer 1976, while checking the nest boxes for small mammals, it became apparent that gypsy moth larvae were using the nest boxes for resting sites and that white-footed mice were using the same boxes for nesting. Not only were predator and prey living together, but gypsy moth larvae survived and adults laid egg masses in the boxes.

Although all the nest boxes were being used by mice, mouse density was low (10 mice per hectare). In

addition to low mouse density, gypsy moth survival appeared to be related to the abundance of cankerworms, a hairless larva whose availability coincided with that of gypsy moth larvae. (In laboratory feeding studies mice have demonstrated a definite preference for hairless larvae; therefore mice in the wild would prefer hairless cankerworms to gypsy moths.)

By 1977, however, the situation in New Jersy had changed. Mouse density had increased to 35 per hectare and the alternative food, cankerworms, had disappeared. Gypsy moth density remained essentially the same, and the larvae continued to rest in the nest boxes, but few gypsy moth larvae survived. The absence of the cankerworms and the increased mouse density appeared to create more competition for food, resulting in lower gypsy moth survival.

Another example of availability and abundance influencing gypsy moth predation occurred in central Pennsylvania in 1975. In early summer gypsy moth larvae began migrating from the tree crown to the litter. At this time, the litter and ground had dried because of limited precipitation and increased sunlight reaching the forest floor through the partially defoliated canopy. This drier condition caused earthworms and other soil invertebrates to move deeper into the soil, making them unavailable as a food source. In this situation, robins began to eat gypsy moth larvae when they became available on or near the ground.

Effect of Defoliation on Gypsy Moth Predators

One factor unique to predators of defoliating insects is the potential effect of defoliation on the predators themselves. Each predatory species chooses a habitat that meets its need for food and cover. A gypsy moth outbreak that results in near or complete defoliation destroys the protective cover and changes the microclimate. Many species of birds rest near the ground (DeGraff et al. 1975), and most forest-dwelling small mammals live on the ground. During the day in a severely defoliated forest, temperature near the ground increases dramatically and humidity drops because of increased evaporation. As species

are subjected to extremes in temperature and humidity and possibly increased predation due to diminished cover, they often leave the area, or they may die.

In 1975 in central Pennsylvania, a population of yellowthroats, a warbler species that prefers brushy habitats, was approximately seven times greater on lightly defoliated plots than on plots that were severely defoliated; the population of redback voles, small mammals that are active day and night, dropped dramatically on severely defoliated plots; the population of white-footed mice continued to increase on all plots (Lautenschlager et al. 1978). Defoliation had less effect on the nocturnal mice than on the voles, which experienced increased ground temperatures during the day and possibly increased avian predation.

Because these conditions occur only at high gypsy moth densities, a time when predators are having little or no effect, it is doubtful that the loss of some predators would have any effect on subsequent gypsy moth population dynamics. In most cases predator populations decrease on the area only for a short time. If refoliation occurs, predator populations increase within 2 to 3 months. However, if major changes occur in stand competition because of defoliation and subsequent tree mortality, and food and cover are affected, significant changes lasting several years could occur in both the density and the variety of predatory species.

The Predator Community

Although research emphasis has been on birds and mammals, some observations have been made of

Figure 4–24.—*Black-billed cuckoo,* Coccyzus erythropthalmus.

predation by other predator groups: Amphibians, reptiles, fish, and several invertebrates. As gypsy moth predators, certain groups are more important than others, and within groups certain species are more important. However, every predator is viewed as part of the predator community, each having a part in the collective effect of predation on sparse prey populations. It cannot be assumed that all gypsy moth predators have been identified, and the roles of identified predators will change as the factors that affect predator potential change.

Birds

The total predator community working together can maintain some gypsy moth populations at harmless levels indefinitely. Birds are a major component of this predator community and play an important role in maintaining low gypsy moth populations.

Many species of birds move to northen areas to raise their young in the spring and early summer and produce two broods of four to six young each. From the time of mating until the young fledge, most birds need a diet high in protein, and insects are eaten extensively. The young themselves seem instinctively to prefer an animal diet to a vegetable diet when given a choice (Welty 1962). Even species that normally do not eat large amounts of insects often become seasonal insectivores.

Birds occur in every habitat type, from nearly open areas to thick brush and mature forests. Within these habitat types they occupy every available stratum, from the litter to the top of the canopy. During the breeding season and in favorable habitats, the total bird community reaches population levels of 5 to 10 pairs per hectare. Research in central Pennsylvania indicated approximately seven breeding pairs per hectare; Holmes and Sturges (1975) in New Hampshire showed roughly 10 pairs per hectare; and Hamilton and Cooke (1940) in New York State indicated approximately five pairs per hectare.

Unfortunately, most observations of birds eating gypsy moths have been made when gypsy moth populations were near or at outbreak levels, a time when predation has no significant effect on gypsy moth populations. From recent observation and from what is known of history, behavior, and predator potential of each species (Forbush and Fernald 1896, Palmer and Fowler 1975, Galipeau 1975), birds can be placed in one of two groups: Those that eat gypsy moths regardless of the gypsy moth density (useful in maintaining sparse gypsy moth populations), and those opportunistic species that are attracted to high-density gypsy moth areas (normally having little or no impact on gypsy moth populations at low densities). The species in the first group are more likely to encounter gypsy moths at low population levels and therefore are more likely to eat them. Birds in the second group will eat gypsy moths in sparse populations if they are encountered.

Bird species in the first group include the black-capped chickadee (*Parus atricapillus*), blue jay (*Cyanocitta cristata*), red-eyed vireo (*Vireo olivaceus*), rufous-sided towhee (*Pipilo erythrophthalmus*), scarlet tanager (*Piranga olivacea*), northern oriole (*Icterus galbula*), catbird (*Dumetella carolinensis*), and robin (*Turdus migratorius*). All the species in this group have characteristics that contribute to their ability to maintain sparse populations at reduced levels: Each eats larvae, pupae, and adult moths, and the chickadee also eats gypsy moth eggs; each is relatively common, and several (chickadee, vireo, towhee, and robin) reach relatively high population densities; most (except tanagers and orioles) have two broods of at least four young per brood per year; and most cover a fairly wide foraging stratum.

Birds in the second group include the yellow-billed cuckoo (*Coccyzus americanus*) and black-billed cuckoo (fig. 4-24) (*C. erythropthalmus*), common crow (*Corvus brachyrhynchos*), chipping sparrow (*Spizella passerina*), starling (*Sturnus vulgaris*), common grackle (*Quiscalus quiscula*), red-winged blackbird (*Agelaius phoeniceus*), and cowbird (*Molothrus atar*). The placement of cuckoos, a species normally associated with outbreaks of hairy caterpillars, in the second group may be questioned; they were placed there because they are uncommon away from a gypsy moth outbreak and normally would not assist in maintaining sparse gypsy moth

populations. A more complete discussion of each species in both predator groups, as well as other gypsy moth predators, can be found in Smith and Lautenschlager (1978).

Many bird species besides those listed above eat gypsy moths. Forbush and Fernald (1896) listed 38 bird species seen eating one or more life stages of the gypsy moth. Many migrating warblers, which often nest farther north, pass through gypsy moth infested areas when the larvae are still small. At resting stops during migration these warblers contribute to the collective effect of bird predation on low-density gypsy moth populations.

Mammals

Mammals, which live in all types of habitats, have perhaps greater predatory impact on sparse gypsy moth populations than any other group of predators. Because of the numbers, distribution, diets, and agility of mammals, most forest insects probably become available prey to them at some life stage of the insect. The impact of mammals is essential if the gypsy moth predator community is to have a regulatory effect.

Mammalian predators of the gypsy moth that have been studied include rodents, insectivores, carnivores, and marsupials. Unlike birds, these predators are not very conspicuous or easily watched because most of them are active only at night. Because mammals are less transitory than birds, they are more stable residents within an area. Unpublished data from central Pennsylvania, south-central Connecticut, and northwestern Connecticut and data from Holmes and Sturges (1975) in New Hampshire and Hamilton and Cook (1940) in New York State indicate that small mammals usually have greater population densities than birds (mammals often reaching 37 to 100 adults per hectare during the spring and increasing sometimes to much higher levels later in the year). These population densities are normally four to eight times higher than birds occupying the same area.

Although mammals have the greatest effect on those insect species that spend part of their developmental period on the ground, they are not restricted to ground level. Squirrels and chipmunks,

which eat many insects during certain seasons (Burt 1957, Hamilton and Cook 1940) are not the only mammals that forage in trees. White-footed mice commonly climb trees to forage (M'Closkey 1975, Horner 1954) and often nest in abandoned woodpecker holes or bird nests.

In an attempt to gain a better understanding of the predator potential of mammals, 15 common woodland mammal species were observed in captivity and in the wild. Study objectives were to identify the major mammalian predators of the gypsy moth, the life stages of the insect that are eaten by each predator, the quantities eaten, and ways to increase the most important predators' effectiveness through management. The mammal species included in these studies were: The white-footed mouse (*P. leucopus*), woodland jumping mouse (*Napaeozapus insignis*), meadow jumping mouse (*Zapus hudsonicus*), flying squirrel (*Glaucomys volans*), shorttail shrew (*B. brevicauda*), masked shrew (*Sorex cinereus*), smoky shrew (*Sorex fumeus*) (fig. 4-25), starnose mole (*Condylura cristata*), striped skunk (*Mephitis mephitis*), raccoon (*Procyon lotor*) (fig. 4-26), and opossum (*Didelphis marsupialis*).

Figure 4-25.—*Smoky shrew,* Sorex fumeus.

Caged mammals were given gypsy moth larvae, pupae, and adults with and without alernative foods. Observations in the laboratory gave no indication that any mammal tested would eat gypsy moth eggs. This was true even when the mammals were given no alternative foods. In several instances, individual mammals would knock the egg masses apart but were never observed eating them. To verify the observations made on these caged mammals, stomachs were examined from snap-trapped small mammals and road-killed raccoons, skunks, and opossums, all collected from within an area of moderate gypsy moth density. Gypsy moth remains were found in the stomachs of all 15 species, revealing that in the wild the gypsy moth is acceptable food and is eaten by all these species.

The White-Footed Mouse and the Shorttail Shrew

After completing analysis of habitats and densities of the mammals and their feeding behavior, it was determined that white-footed mice and shrews have significantly greater predator potential than other

Figure 4–26.—*Raccoon,* Procyon lotor.

species. White-footed mice have demonstrated their ability to eat large numbers of gypsy moths in the wild. After an intensive study of the gypsy moth predators, the white-footed mouse is recognized as the single most important predator of the gypsy moth.

A small mammal food preference study (conducted in the wild with healthy gypsy moth pupae placed in the litter) indicated that white-footed mice and shrews (both shorttail and *Sorex* sp.) each accounted for approximately 50 percent of the gypsy moth mortality attributed to small mammals. Remains of gypsy moth larvae and pupae have been found in both stomachs and droppings of shorttail shrews collected within a sparse gypsy moth population.

White-Footed Mouse (*Peromyscus leucopus*)

The white-footed mouse has received more attention than any other predator of the gypsy moth. Recent studies have emphasized the beneficial impact of this mammal in sparse gypsy moth populations (Campbell and Sloan 1976, 1977a), their feeding behavior, and even the possibility of managing this species (Smith 1975) to increase its effectiveness against the gypsy moth.

This mouse (average weight about 20 g) is the most common and most widely distributed small mammal in the Northeast. It lives in a wide variety of habitat types, from suburban lawn edges to mature forests, but is most abundant in thick brushy areas. Under ideal conditions, spring breeding densities can reach 13 mice per hectare, with densities later in the year reaching two to three times that number. Each breeding season, adult female mice produce an average of three litters of approximately four young per litter.

White-footed mice have remarkable agility and thoroughly explore and utilize their habitat, nesting and foraging from below the ground to tree tops. Their diet consists of a variety of seeds, nuts, and insects, and their feeding behavior is truly opportunistic. Insects form a substantial part of the mouse's diet in the spring and summer (Martin et al. 1961), so it is not surprising that it preys on the gypsy moth when available.

The mouse has an interesting way of eating late-instar gypsy moth larvae. It grasps the insect with its forepaws and pulls back the head capsule with its teeth, discarding it along with the bright-green upper digestive tract. While rolling the insect back, just as a sock might be rolled, the mouse eats the body fluids and some internal membranes (Smith and Campbell 1978). In the woods, larval remains are most likely to be found at the base of host trees but have been found 9 m up in trees.

The process of rolling back the larval skin has to be learned; young mice usually have an unsuccessful first encounter with larvae. A mouse will sniff a larva, as it does other foods, but if it gets too close, the larval hairs become embedded in the mouse's nose. The mouse's second approach is more cautious, and soon the larva is a regular part of its diet.

White-footed mice usually eat pupae by opening one end and eating the contents, but many times they will simply tear apart pupae and eat them. The mice will select female pupae over the smaller males, but both are eaten. Mice do not store pupae, as they do some other foods. Gypsy moth adults (male and female) are also eaten by the white-footed mouse. After capturing a moth, the mouse eats the entire body, normally leaving the wings and legs. The mice, which apparently do not eat gypsy moth eggs, do eat the abdomen of the adult female, including the eggs within.

The number of insects eaten by an animal in captivity does not necessarily indicate how many that animal will eat in the wild, but it can provide a rough idea of the maximum number that could be destroyed over a certain time period. In captivity, white-footed mice with alternative foods (apples and mouse chow) have eaten as many as 46 of 50 fifth-instar larvae over 24 hours. Mice without alternative food have eaten 50 of 50 fifth-instar larvae. Mice have eaten as many as 20 of 40 pupae when an alternative food was available, and 28 of 40 when no alternative food was provided. Overall, mice ate an average of 90 percent of the larvae offered when no alternative food was provided. Alternative foods had no effect on the number of pupae eaten; in both cases, when pupae were offered

with and without an alternative food, mice ate an average of 30 percent of the pupae.

Shorttail Shrew (*Blarina brevicauda*)

The shorttail shrew is the largest North American shrew (average weight about 18 g) and is the most common insectivore in many areas. It is common in a variety of habitats (forests, grasslands, marshes, and brushy areas) east of the Rocky Mountains. Although abundant (two to three litters of five to eight young per litter per year, with populations reaching 20 per hectare), shorttail shrews are among the least conspicuous of the forest mammals. They are seldom seen but can be heard rustling through the litter as they search for food beneath logs, stumps, rocks, and leaf litter, and even in the surface runways of rodents—all places where gypsy moth larvae rest and pupate. In addition to worms, snails, and other small animals, nearly half the shorttail shrew's diet consists of insects (Palmer and Fowler 1975). As Burt (1957) states, "As an insect destroyer, this shrew, because of its abundance, undoubtedly is of considerable economic importance."

A shorttail shrew eats a gypsy moth larva by grasping the larva with its forepaws, holding the insect against the ground, rolling it over, and biting it near the middle segment on the ventral side. The bite injects a poison secreted from the glands in its mouth that helps to subdue the larva. The shrew then eats the head. Seldom will the head capsule remain; this is one way to distinguish larvae eaten by shrews from those eaten by mice. The shrew then eats the larva, including the gut from the ventral side. All that remains is the skin from the back, where the hairs are attached.

Caged shorttail shrews consistently ate at least 65 percent of the larvae offered and nearly all the pupae, even when an alternative food (canned dog food) was available. Shrews ate large numbers of pupae but, unlike mice, have not shown a preference for the larger female pupae over the smaller males. A shrew will usually pick up a pupa and carry it below ground to a nest. One adult shorttail shrew was seen making 15 separate trips to its nest, carrying one pupa each time.

Amphibians, Reptiles, and Fish

When the role of vertebrate predators of the gypsy moth is discussed, the emphasis is placed on birds and mammals. However, the following amphibians, reptiles, and fish have been observed eating gypsy moth larvae and/or adults: American toad (*Bufo americanus*), Fowler's toad (*Bufo woodhousei fowleri*), wood frog (*Rana sylvatica*), tree frogs (*Hyla versicolor* and *H. chrysoscelis*), green snakes (*Opheodrys aestivus* and *O. vernalis*), garter snake (*Thamnophis sirtalis sirtalis*), hognose snake (*Heterodon platyrhinos*), fence lizard (*Sceloporus undulatus*), brook trout (*Salvelinus fontinalis*), largemouth bass (*Micropterus salmoides*), and the bluegill (*Lepomis macrochirus*).

Little is known about this group's role as predators of the gypsy moth or other forest insects. Because these vertebrates are coldblooded, their metabolic rate is normally much lower than that of birds and mammals; this lower metabolic rate requires less food and thus their potential for consuming prey is low. It is doubtful, therefore, that these vetebrates have a significant impact.

Movement of prey appears to be the key to food selection (Curio 1976) by the amphibians, reptiles, and fish that have been observed. This selection is largely a random process, with foods taken in proportion to abundance and availability. The American toad (fig. 4–27) was noted as a predator of the gypsy moth as early as 1896 by Forbush and Fernald, when they reported that these toads ate great

Figure 4–27.—*American toad,* Bufo americanus.

numbers of gypsy moth caterpillars in infested brushlands. They examined the stomachs of three toads taken in that area and found them to contain 7, 15, and 65 gypsy moth caterpillars, respectively. Unfortunately, when any pest insect becomes abundant enough to represent the major portion of the diet of this group, it is unlikely that predator pressure from even the entire predator community, including birds and mammals, will have an impact on the pest population. During these periods of prey abundance there are just too many insects for the predators to eat.

Invertebrate Predators

In comparison to vertebrate predators, little is known about the invertebrate predators of the gypsy moth. Perhaps the exotic ground beetle, *Calosoma sycophanta*, has received more attention than any other invertebrate predator. *C. sycophanta* was imported from central Europe and released (1906–26) in New England to help control the gypsy moth. Also, during the 1960's and early 1970's, *C. sycophanta* adults and larvae were collected from gypsy moth outbreak infestations in the Northeast and released in New York, New Jersey, Pennsylvania, and Vermont (Reardon 1978). Although abundant only in certain locales, this beetle is now well established in infested areas. It is usually observed, however, only when there is an abundance of gypsy moths. Despite its high attack potential (Burgess 1929) and efforts to import and release this predator, it remains questionable what impact these beetles have within low-density gypsy moth populations. Campbell (1975) reported one instance where "Gypsy moth numbers had been high for several years running, and the [*Calosoma*] beetles finally caught up numerically and caused heavy mortality among their prey."

More recently, a pentatomid, *Dinorhynchus dybowskyi*, a predator of gypsy moth larvae, pupae, and adults in Japan, has been imported to study the feasibility of establishing it in this country. The insect is currently in quarantine.

Although exotics have been studied extensively, recent study on the effect of small-mammal selective feeding behavior on gypsy moth pupae brought to light that certain native invertebrates, namely carpenter ants (*Camponotus pennsylvanicus* and *C. ferrugineus*) and harvestmen (*Leiobunum longipes* and *L. politum*), appear to have considerable predator potential. Until this study, the potential of these native invertebrates had received little attention because of the emphasis on small mammals.

Some other invertebrates identified as gypsy moth predators include: Other ground beetles (*Calosoma calidum*, *C. scrutator*, and *C. frigidum* (fig. 4–28), and *Carabus* sp., *Agonum* sp., and *Harpalus* sp.), a stink bug (*Podisus maculiventris*), the bald-faced hornet (*Vespula maculata*), and the praying mantis (*Mantis religiosa*). Even mites are known to destroy gypsy moth eggs.

Although the emphasis should be on the vertebrates, ignoring invertebrates will lead to some loss of appreciation and understanding of the importance of vertebrate predators. It is therefore essential to examine all the components of predation in order to assess the value of any predator or group of predators within a community.

Forbush and Fernald (1896) listed 11 species of spiders from six families that they had seen attacking gypsy moth eggs, larvae, pupae or adults. It is known that spiders occur in such numbers and occupy such diverse habitats that they have the potential to kill significant numbers of certain forest pest insects. Juillet (1961) indicated that spiders appear to be the most effective predator of the European pine shoot moth. Renault and Miller (1972) state that spiders might play a significant role in determining the mean endemic density of budworm. Furuta (1977) evaluated two species of hunting spiders, *Oxyopes sertatus* and *O. badius*, as a mortality factor of gypsy moth. Furuta reported that although mortality caused by the predation was negligibly small, and could not control the local occurrence of the gypsy moth, when small numbers of larvae were on a tree they would be exterminated. The role of spider predation as a group remains unclear and warrants further investigation.

Invertebrate predators appear to be more important than was previously thought. Campbell

and Sloan (1976), when assessing gypsy moth mortality factors, assumed that missing pupae had been carried away or eaten by vertebrates. However, recent field observations indicate that many of the missing pupae classified as killed by vertebrates could actually have been killed and removed by invertebrates.

In a field experiment, ants and harvestmen (fig. 4-29) removed a substantial portion of the pupae (both parasitized and healthy) that were placed under small wooden shelters in the litter or that were allowed to spin up and pupate in those shelters. Wounds in pupae attacked by invertebrates had finely serrated edges, which easily distinguished them from those attacked by small mammals. A peppering effect, caused by pupal case remains left on the boards, also indicated invertebrate activity; on litter this peppering would have gone unnoticed. Invertebrates removed a substantial number of gypsy moth pupae. However, whether the invertebrates were acting as scavengers or predators or both is still being studied.

The effect invertebrate predators can have has been demonstrated on several agricultural crop pests. Within the complex forest ecosystem it is difficult to measure invertebrate predatory effect on gypsy moths. Furthermore, two problems arise when trying to include invertebrate predators in an integrated pest management system. Invertebrate predators are not abundant in all areas, and they are often dependent on the density of their prey. Even within these limits, however, they can have a significant impact, as in the case reported by Campbell (1975), when *Calosoma* beetles caused heavy mortality to a gypsy moth population in Connecticut.

Measuring Predator Impact

Although it is popularly believed that predation can play a significant role in the dynamics of many forest

Figure 4-28.— *Ground beetle,* Calosoma frigidum.

insects, few studies have shown the precise role of these predators. Perhaps the most difficult task in evaluating predators is assessing their effect.

Despite recent research efforts resulting in better understanding of the components and interactions of the gypsy moth predator/prey system, any discussion of predator impact at this time will be incomplete, because of the scarcity of data pertaining to the effects of alternative foods on the rate of predation on all gypsy moth life stages. The significance of reported mortality percentage rates, such as vertebrate predators killing about 70 percent of the pupae (Campbell and Sloan 1976), or the mortality percentage from current studies (discussed in following paragraphs) will remain inconclusive until gathered simultaneously with sufficient data on abundance and availability of alternative foods. The accuracy of these percentages is not questioned but rather the relationship of these predation percentages to the previous year(s) or subsequent year(s) predation percentages. To better relate to the population dynamics of the insect, mortality percentages should not only span a sufficient time but should also include an analysis of the abundance and availability of alternative foods.

One example of how alternative foods can affect gypsy moth predation rates is the previous reference to *P. leucopus* in New Lisbon, N.J. The year cankerworms were abundant (1976), very little gypsy moth mortality could be attributed to *P. leucopus*. During that year, any study designed to measure *P. leucopus* predatory impact on that gypsy moth

Figure 4-29.— *Harvestmen*, Leiobunum *sp.*

population would have grossly underestimated the value of this important predator. Conversely, an analysis of *P. leucopus* impact on the gypsy moth population in the same area the following year (1977), when cankerworms were not available and high predation by *P. leucopus* was observed, could have overestimated the value of this predator.

Gypsy moth survival is often a function of the resting site they select (Campbell et al. 1977, Campbell et al. 1975*a, b*). Some sites offer much higher survival potential than others. However, the diversity of predators and the niches they occupy leave no entirely safe refuge for this insect. Actually, the predator community collectively has the potential to eat all the gypsy moths within a sparse moth population, but this is never likely to occur. Gypsy moths are never the only food source. The abundance and availability of alternative foods vary annually, as does the consumption rate of each food. Also, each predator's preferences for the available foods will influence the potential impact any predator can have on any prey species.

Certain aspects of the role of gypsy moth predators are fairly well understood, while many others have not yet been determined. This is probably best exemplified by research experience. The available information in the early 1970's led to the belief that when significant predation of gypsy moth pupae in sparse populations occurred it was contingent upon white-footed mouse activity. It was then hypothesized that through management to increase populations, this important predator might be made even more effective.

In one study, supplemental food and/or nest boxes were provided during the winter for two populations of *P. leucopus* to reduce overwintering mortality. (A complete description of that study is presented in Smith 1975.) The objective was to increase the spring breeding density that would result in a greater mouse density in June and July, the time when gypsy moth larvae and pupae would become available as food to these predators. Data presented in table 4–16 and figure 4–30 show that populations of this important predator can be managed. The provision of nest boxes did not significantly (χ^2, $P>0.05$) increase mouse

density in June and July when the following comparisons were made: Control vs. nest boxes only, and feed vs. feed plus nest boxes. Although in both situations nest boxes had a positive effect on mouse density, the abundance of natural nesting sites, stone walls, stumps, etc., in the 80-year-old mixed hardwood stand prevented a significant correlation. Conversely, supplemental feed, in both the feed-only and the feed-plus-nestbox plot did significantly (χ^2, $P<0.005$) (table 4–16) increase mouse density in June and July.

In figure 4–30, site one received supplemental feed while site two did not. The percentage of increase of *P. leucopus* for June 1974, 1975, and 1976 was 56, 52, and 106, respectively, and in July (same years), 60, 77, and 40. Although the addition of nest boxes caused no significant change in mouse density, the treatment allowed for more complete analysis of the effect of supplemental food. Use of nest boxes by age class was proportional to age-class distribution within the population (based on census), if young with adult females were eliminated. Litter size was not

Table 4–16.—*Number of* P. leucopus *per type of treatment area (1.7 ha) observed during the selected time periods*

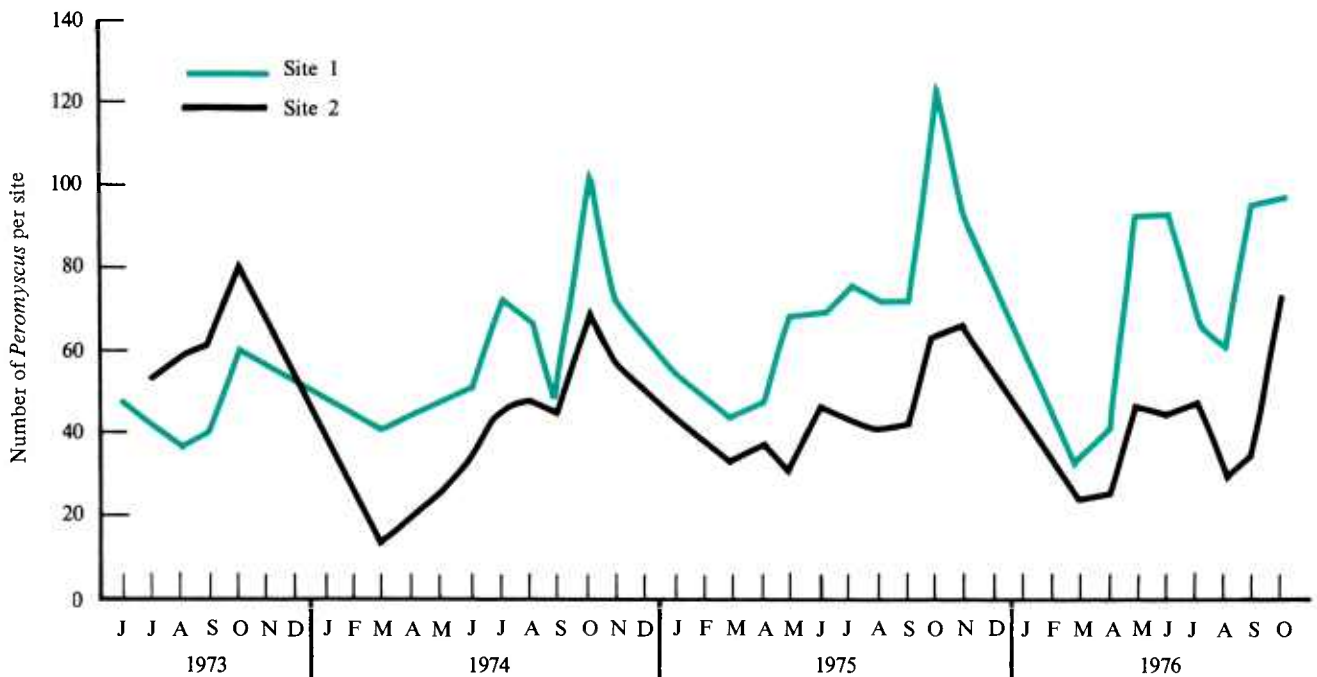|		Control	Nest boxes	Feed	Feed and nest boxes
		(Spring breeding population, March and April)			
1974		12	6	36	18
1975		23	26	26	35
1976		14	18	22	26
	Total	49	50	84	79
		("Target period" population, June and July)			
1974		27	33	40	45
1975		26	36	51	46
1976		31	34	48	56
	Total	84	103	139	147
		(Fall population, September and October)			
1974		32	41	45	40
1975		23	40	39	71
1976		27	22	62	66
	Total	82	103	146	177

Figure 4-30.—*Population curves of* P. leucopus
showing the effect of supplemental feeding. Site 1
received food; site 2 did not.

significantly affected ($P>0.05$) in fed vs. unfed plots; however, figures 4-31 and 4-32 show the effect feeding had on the period of reproduction. After feeding, juvenile white-footed mice in litters were observed from April through November. During the study, juveniles in litters were observed in 17 months in the fed area (site one), compared to only 10 months in the unfed area (site two). Feed did extend the period of reproduction, with some reproduction occurring in November each year in the fed area (site one) (including 1976, not shown in fig. 4-31), while none was observed in site two. However, more significant was the more continuous reproduction during the breeding season in site one.

The results of this study demonstrated that it is biologically feasible to increase *P. leucopus* populations. Following initial treatment in the fall of 1973, the fed population density exceeded the unfed population throughout the duration of the study. However, a number of factors remained to be considered: The economics of bringing about the change, the effects of increased densities on the forest,

and the possibility that this increased density could predictably increase predation of gypsy moths. Therefore, it seemed that a logical step was to measure the impact of the increased mouse density on the survival of gypsy moths. In 1976, three studies were designed to determine the feeding behavior of white-footed mice in relation to healthy and parasitized male and female pupae placed in the litter.

Methods and Procedure

The study area was located on the Lake Saltonstall reservoir watershed in Branford, Conn. Three sites were located in a 65- to 85-year-old stand of mixed hardwoods, with the overstory composed chiefly of oak (*Quercus* sp.), hickory (*Carya* sp.), and black birch (*Betula lenta*). The shrub layer was dominated by maple-leaved viburnum (*Viburnum acerifolium*), arrowwood viburnum (*Viburnum dentatum*), and spicebush (*Lindera benzoin*). The herb layer was dominated by maple-leaved viburnum, virginia creeper (*Parthenocissus quincifolia*), and poison ivy
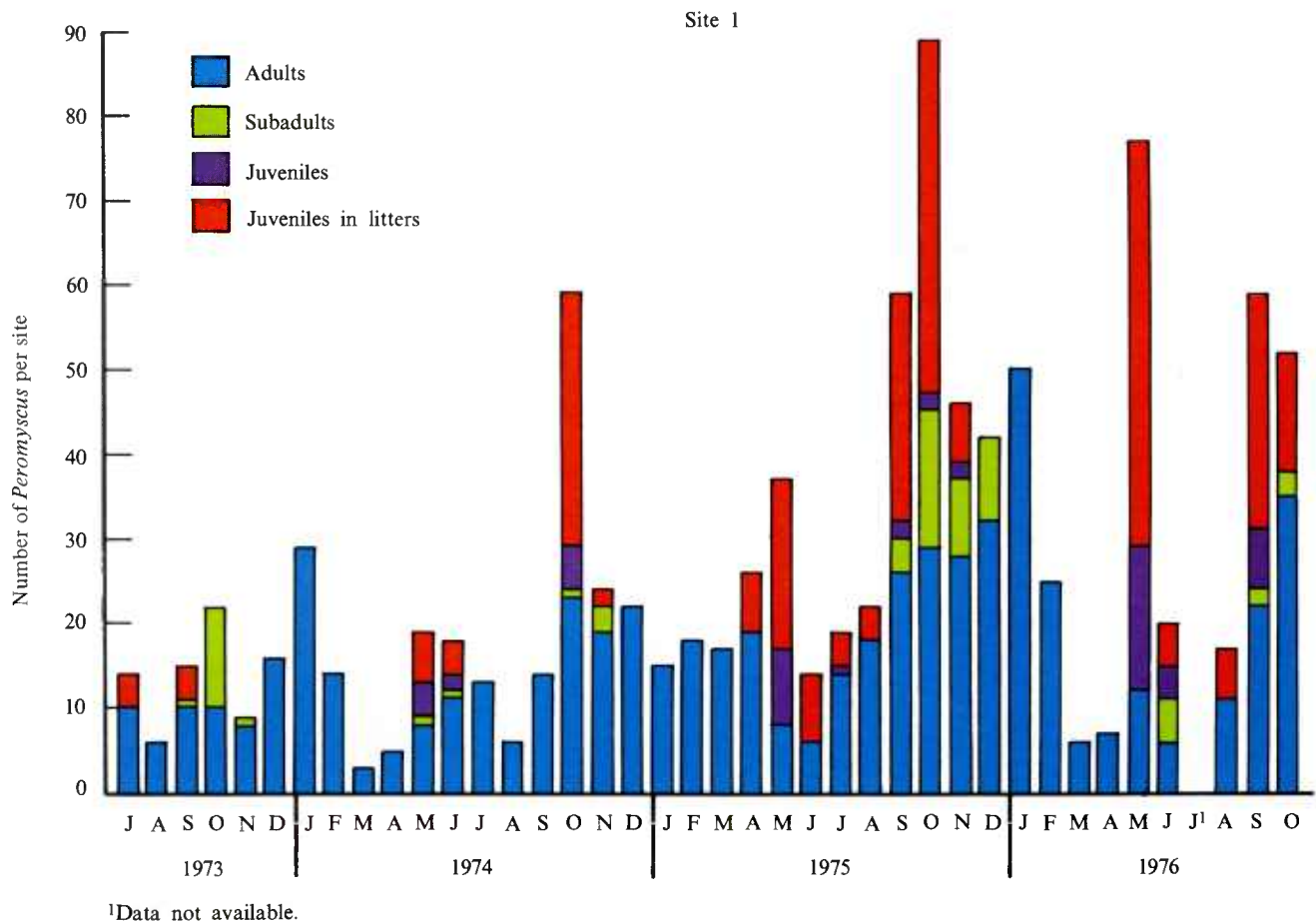
Figure 4-31.—*Age structure of the* P. leucopus *population that received supplemental food.*

¹Data not available.

(*Rhus radicans*). Each site bordered the access road that paralleled the east side of the reservoir. The sites were of similar slope with a westerly aspect and were approximately 15 m above sea level. Sites one and two were each 3.3 ha; site two was located 2 km north of site one. A third site of 2.5 ha was located 230 m south of site one.

During the time of these studies, naturally occurring gypsy moth populations on the study area were extremely sparse (only a few gypsy moths were observed).

Small-Mammal Census

To census the small-mammal populations, Sherman® live traps 7.5×7.5×30 cm were set on grids at 15 m intervals. Grids in sites one and two were 12×12, and the grid in site three was 9×14. Traps placed on these grids were baited with a peanut butter/oatmeal mixture and provided with dry cotton for nesting. A 5-day small-mammal census was taken 1 week before the placement of pupae in these sites. During the census, each trapped small mammal was identified by toe clipping (Blair 1941), and its sex, age, condition and the trap number were recorded.

Study 1: Food-Preference Study

This was designed to determine the selection preference by *P. leucopus* between male and female and healthy and parasitized gypsy moth pupae. At
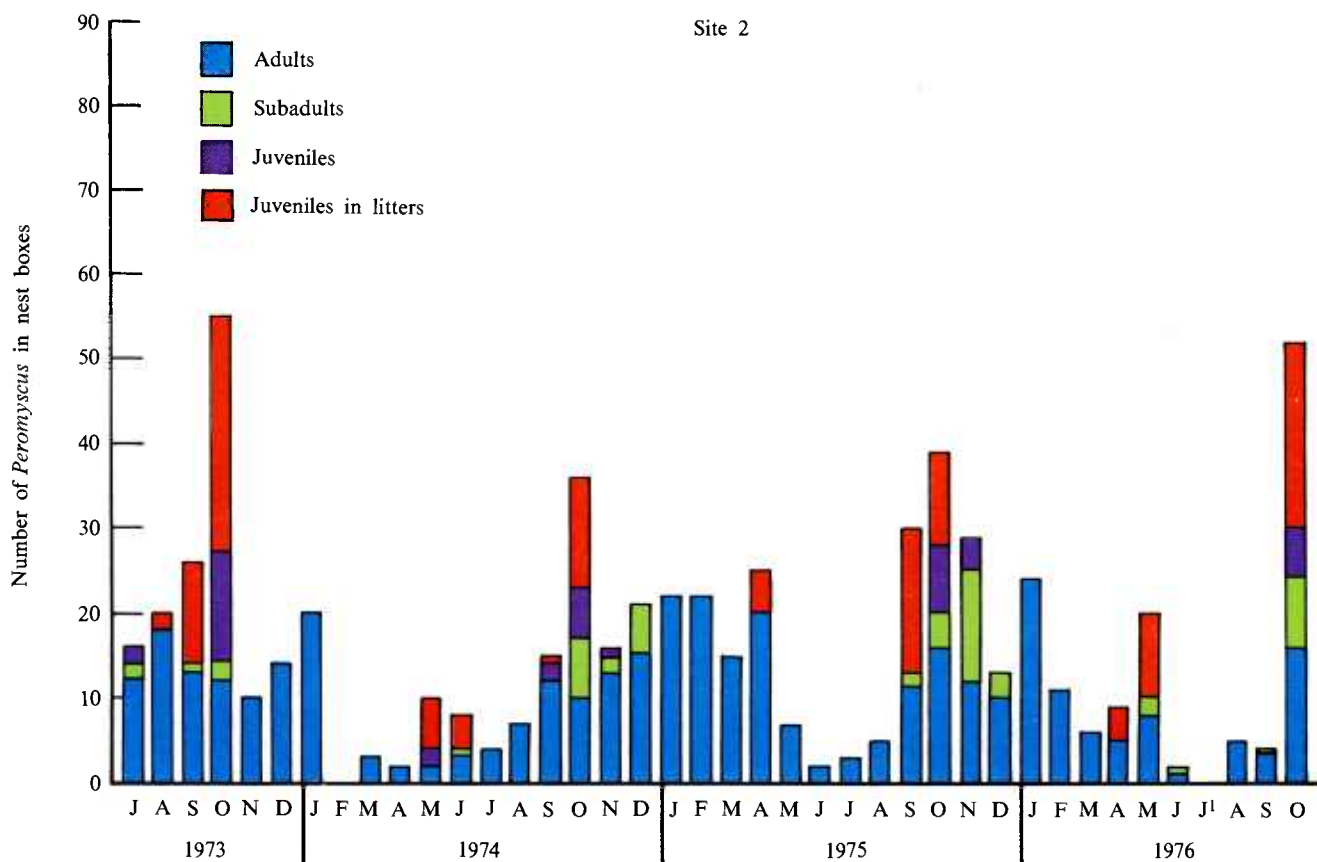
Figure 4-32.—*Age structure of the* P. leucopus
population that did not receive supplemental food.

each grid location within each site, items presented for potential predation included one healthy male pupa, one healthy female pupa, one *Blepharipa pratensis* parasitized male pupa, one *B. pratensis* parasitized female pupa, one *B. pratensis* puparia, and a drop of beeswax (control).

Each test item was attached to a 0.6×15×20 cm masonite platform with a drop of beeswax. The above food items and the wax were arranged in a 10-cm diameter circle on each platform. The location of each pupa type and the beeswax on each board were selected at random. Locations were then permanently marked on the boards to aid in replacing eaten pupae. Pupae that were eaten were replaced daily. A protective canopy consisting of two boards measuring 2×15×30 cm, nailed lengthwise to form a right angle,

was placed over each pupal board. In order to exclude birds from the pupal boards, poultry netting was stapled to each end of the protective canopies. The healthy pupae and fourth- and fifth-instar larvae used in this study (and studies two and three) were supplied by the Otis Methods Development Center, Otis Air Base, Mass. Fourth- and fifth-instar larvae were parasitized by *B. pratensis* and reared to pupation at the Forest Service laboratory in Hamden, Conn. Parasitism was verified by the X-ray technique described by ODell et al. (1974).

On July 16, 1976, the platforms with the treatments and the protective canopies were placed on the appropriate grid locations in the study areas. Beginning 8:30 a.m. the following morning, the platforms were examined, the condition of each item

recorded, and eaten items replaced. The procedure was repeated for the next 21 days.

Study 2: Predation of Naturally Spunup Pupae

This was designed to examine the survival of pupae (from pupation to emergence) and was conducted without replacement of eaten pupae, which would have created a "feeder type" situation.

Fifth- and sixth-instar larvae (30–35 individuals) and artificial diet (ODell and Rollinson 1967) were placed in nest boxes formed by tightly taping two canopies and two boards together. The insects were allowed to spin up in these configurations approximately 1 week before the canopies and platforms were placed in the study area. Four combinations of canopies and boards were used at each location: (1) A board with naturally spunup pupae and pupae placed in wax (for each naturally spunup male and female pupae one pupae of the same sex was attached to the board by beeswax as described in study 1); (2) a board with pupae covered by a canopy containing no pupae; (3) a board and a canopy both with pupae; and (4) a canopy with pupae.

When the tape was removed, naturally spunup pupae were attached to several of the canopies and platforms that had formed these boxes. Larvae that had not pupated and uneaten diet were removed from the canopies and platforms. One hundred canopies (four categories combined) were randomly assigned individual grid points and placed within one study area. Data collection began August 5, 1976, with each canopy and platform checked daily until all pupae had been classified as dead, missing, eaten, or emerged. Pupae removed or missing were not replaced.

Study 3: Invertebrate Study

This was designed to determine the contribution of invertebrates to the predation of and/or scavenging of healthy, parasitized, and injured pupae. At 18 locations each for sites one and two, 36 vertebrate exclosures were established to determine the differential selection of invertebrates of healthy male pupae, healthy female pupae, pinpricked male pupae,

pinpricked female pupae, *B. pratensis* parasitized male pupae, *B. pratensis* parasitized female pupae, *Brachymeria intermedia* parasitized male pupae, and *B. intermedia* parasitized female pupae.

Pupae were attached to platforms with a drop of melted beeswax. One of each of the above types of pupae was then placed on each pupae location platform at locations determined at random. These predetermined locations were permanently marked on each platform to aid in replacing killed or missing pupae. Vertebrate exclosures consisted of wood and wire mesh. Outside dimensions of the exclosures measured 8×24×25 cm, and wire mesh (0.6 cm) covered two sides and the bottom of these exclosures. The other two sides consisted of a board measuring 2×7.6×25 cm. A piece of plywood 0.6×24×25 cm formed the lid. The lid was hinged to the back and had a latch in the front to allow opening and closing of the exclosures. Killed and missing pupae were replaced daily.

On July 21, 1977, the exclosures were placed on a 3×6 grid 30 m on center in the two study areas. Exclosures were examined daily, and data were recorded for 37 consecutive days. On the 18th day, a strip of wire mesh approximately 1 cm wide was cut and removed the length of two sides on every other exclosure. After the strip of wire mesh was removed, these boxes allowed access to large invertebrates and small vertebrates (small and young shrews and young mice) and were referred to as "open" (tables 4–17, 4–18, and 4–19).

Results and Discussion

A census of *P. leucopus* taken prior to the start of these studies indicated a density of approximately 20 mice per hectare, assuming a "draw" of mice from outside the trapping grid of 2.9 m (Gentry et al. 1971).

Experience has shown that the live-trap census technique used to determine *P. leucopus* density is a poor indicator of shrew density. Although three species of shrews—*B. brevicauda, S. cinereus,* and *S. fumeus*—were captured, valid estimates of their densities were not possible. For shrews, pitfall traps provide high capture efficiency (Pucek 1969), and they are being used in the 1978 field season.

Table 4-17.—*Daily mean percent and number of pupae eaten, examined over 27 days*

Observation time and board condition	Type of pupae				
	Healthy males	Healthy females	Healthy males and females	*Brachymeria*-parasitized males and females	*Blepharipa*-parasitized males and females
Day 1–9 (closed)	2.07 (531)	4.33 (531)	3.20 (1062)	0.85 (1060)	2.87 (699)
Day 10–16 (closed)	1.59 (251)	6.02 (249)	3.81 (500)	1.98 (504)	10.27 (487)
Day 17–27 (closed)	2.50 (160)	11.25 (160)	6.88 (320)	3.70 (324)	12.30 (309)
Day 17–27 (open)	20.25 (158)	29.11 (158)	24.68 (316)	29.02 (317)	34.27 (286)

Table 4-18.—*Daily mean percent and number of pupae and prepupae eaten, examined over 7 days with replacement*

Board type and sample size	Type of pupae								
	Healthy males	Healthy females	*Brachymeria*-parasitized males	*Brachymeria*-parasitized females	*Blepharipa*-parasitized males	*Blepharipa*-parasitized females	Pin-pricked males	Pin-pricked females	Prepupae
Open (n)	22.22 (144)	33.34 (144)	33.34 (144)	30.55 (144)	—	—	23.59 (144)	55.83 (142)	62.77 (81)
Closed (n)	6.95 (144)	15.28 (144)	2.78 (144)	5.56 (144)	7.69 (64)	24.46 (55)	11.11 (144)	16.67 (144)	10.55 (81)
Difference between open and closed	15.27	18.06	30.56	24.99	—	—	12.48	39.16	52.22

Table 4-19.—*Eight-hour mean percent and number of pupae and prepupae eaten, examined at 8-hour intervals for 3 days*

Board type and examination time	Type of pupae			
	Healthy males and females	*Brachymeria*-parasitized males and females	Pinpricked males and females	Prepupae
Open, midnight (4–12 p.m.)	6.25 (144)	6.94 (144)	11.11 (144)	0 (16)
Open, 8:00 a.m. (12–8 a.m.)	9.26 (108)	10.19 (108)	8.55 (108)	0 (15)
Open, 4:00 p.m. (8 a.m.–4 p.m.)	1.85 (108)	2.78 (108)	4.63 (108)	9.09 (11)
Closed, midnight (4–12 p.m.)	.70 (144)	.69 (144)	4.17 (144)	0 (19)
Closed, 8:00 a.m. (12–8 a.m.)	2.78 (108)	1.85 (108)	1.85 (108)	5.26 (19)
Closed, 4:00 p.m. (8 a.m.–4 p.m.)	.93 (108)	1.85 (108)	2.78 (108)	0 (15)

The original intent, based on available predator/ prey information when study 1 was designed (which assumed *P. leucopus* to be the only significant predator of pupae), was to determine the following for *P. leucopus:* The selection of healthy and parasitized pupae, rate of predation of pupae in a natural population of mice, and the rate of predation in a managed population. After only a few days it became apparent that a realistic analysis of predation could not be viewed in perspective with a single predatory species. In retrospect it was admittedly overly optimistic to believe that the precise impact of the mice on the pupae could be determined. By the end of the field season it was hard to determine, without the aid of a computer to assist in analysis, whether mice, shrews, or even invertebrates (ants and harvestmen) had eaten more pupae. During that study it became apparent that little was known about the components and interactions within the total gypsy moth predator community.

Table 4–20 combines and summarizes the preferences for the five food choices presented during study 1. Predators observed daily eating pupae were ants, including black (*Camponotus pennsylvanicus,* fig. 4–33), and red (*C. ferrugineus*) carpenter ant workers and several species of harvestmen, including *L. longipes* and *L. politum.*

Vertebrate predation was identified by "sign" such as incisor marks in the wax, feces on the platform, the size and shape of the pupal fragments, or the manner in which pupae were opened. These types of signs were sufficient to separate *P. leucopus* predation from shrew predation. Since sign often indicated that both mice and shrews had visited the same feeding platform, it was not possible to determine the amount of predation or specific preferences for various items between mice and shrews. Compounding this problem was invertebrate feeding activity, which resulted in many pupae being attacked and eaten. Invertebrate feeding sign was easily distinguished from that of vertebrates by the finely serrated edges and the small pupal fragments that left a "peppering" effect on the platforms. Table 4–20 shows the selective

Table 4–20.—*Predator selection of gypsy moth pupae and* Blepharipa *puparia placed on the litter*

	Total number available	Total number remaining	Percent survival[1]
Healthy male	4189	2041	48.7
Healthy female	4184	1840	43.9
Blepharipa-parasitized male	4187	768	18.1
Blepharipa-parasitized female	4187	616	14.7
Blepharipa puparia	4163	1060	25.0

[1]Mean survival based on 24-hour disappearance, with replacement.

preference and average predation rate for each category during 24 hours. Within both the healthy and parasitized categories female pupae were more likely to be eaten than male pupae (x^2, $P<0.005$). This intraspecific selection appeared to be a function of prey size, a phenomenon noted by Holling (1958)— female gypsy moth pupae are approximately twice the size of males. However, it is questionable whether this difference in selection, although statistically significant, would be of significant magnitude, biologically, to affect the dynamics of the gypsy moth. This differential selection does not approach the level reported by Campbell and Sloan (1976).

Table 4–20 also demonstrates interspecific selection. *B. pratensis* puparia, which are considerably smaller than either male or female gypsy moth pupae, were selected three to one over gypsy moth pupae. Perhaps most significant in table 4–20 is the fact that the predator community discriminated between healthy and parasitized pupae. Gypsy moth pupae (both sexes) parasitized by *B. pratensis* were subject to approximately three times ($P<0.005$) the predation rate of healthy pupae. It had been previously reported (Campbell and Sloan 1977*a*, Furuta 1976) that many parasites are inadvertently destroyed by predators. However, these observations show that parasitized pupae were actually preferred. Apparently, gypsy moth pupae parasitized by *B. pratensis* become more vulnerable to invertebrates. The mature (last instar) *B. pratensis* larva appears to distend the

gyspy moth pupa, exposing the intersegmental tissues, which become the foci of attack for most of the invertebrates.

Whereas study 1 focused on predator preferences, given that each item tested had equal abundance and availability, study 2 focused on survival of pupae from time of pupation until adult emergence. The results of this study, which used naturally spunup pupae, demonstrated the responsiveness and opportunistic behavior of the predator community that contributes to the suppressive forces of predation. In study 2, nearly 500 late-instar larvae were allowed to pupate in canopies that were then placed in the litter. The amount of predation in these canopies allows for an accurate determination of percent survival from pupation through emergence, and the percent killed by predators.

In study 2, predators killed over 70 percent of the pupae in the four test categories (table 4–21): 25 percent of the pupae survived. These percentages should not be used to define or evaluate predation potential for other populations, because even within natural populations predation rates vary from site to site. More importantly, these observations as well as those of Campbell and Sloan (1976, 1977a) are for specific predator and prey populations at a specific time. However, the underlying principles of predator/prey interactions, seen in these studies, can be applied from one site to another.

The data presented in tables 4–17, 4–18, and 4–19

Figure 4–33.—*Black carpenter ant,* Camponotus pennsylvanicus.

Table 4-21.—*Total predation of naturally spunup pupae, measured from pupation to emergence*

Board type	Number of boards	Pupal location	Total number	Number dead	Percent	Number emerged	Percent	Number removed (predation)	Percent
1	27	Board	114	4	3.50	26	22.81	84	73.68
2	14	Board	110	3	2.73	36	32.73	71	64.54
3	14	Board	45	3	6.67	1	2.22	41	91.11
3	14	Canopy	52	3	5.77	15	28.85	34	65.38
4	45	Canopy	157	4	2.55	43	27.39	110	70.06
Total			478	17	3.56	121	25.31	340	71.13

indicate the relative contribution by invertebrates to the predation that occurred in studies 1 and 2 and provide insight into the importance of invertebrates in the population dynamics of the gypsy moth.

By day 17 (study 3) it became apparent that the larger phalangids often seen eating pupae under the canopies did not have access to these boxes through the smaller sized mesh. Therefore, on day 17 every other box was "opened" as described (see Methods). This resulted in an immediate and dramatic increase in predation rate (table 4-17). This opening allowed not only the larger invertebrates (including phalangids and ground beetles) access to the box but also *Sorex* sp., young *B. brevicauda,* and young *P. leucopus.* An examination of the data before and after opening (table 4-17) allows a partial view of the partitioning of predation on gypsy moth pupae by the predator community. Predation by the vertebrates and/or large invertebrates was significantly greater ($P<0.005$)—from two to eight times that of the small invertebrates. However, the supportive role of the small invertebrates in gypsy moth predation is demonstrated for the first time.

Table 4-17 shows that invertebrate predators preferred healthy female pupae to healthy male pupae (χ^2, $P<0.005$), preferred pupae parasitized by *B. pratensis* to pupae parasitized by *B. intermedia* (χ^2, $P<0.005$), and preferred healthy pupae to pupae parasitized by *B. intermedia* (χ^2, $P<0.005$).

Are invertebrates acting more like scavengers or predators? Table 4-17 shows that the answer will vary depending on their choice. Invertebrates ate significantly (χ^2, $P<0.005$) less healthy pupae (4 percent) than the *B. pratensis* parasitized pupae (7 percent), so in this case they acted more like scavengers. However, healthy pupae were preferred (χ^2, $P<0.005$) to *B. intermedia* parasitized pupae (table 4-17), so when that comparison is made, invertebrates were more predatory.

Comparison of preferences for pupae and prepupae is presented in table 4-18. Across all categories, small invertebrates (closed exclosures) accounted for approximately 28 percent of the predation (range 8–47 percent) on a daily basis, while large invertebrates and the small invertebrates accounted for the remaining 72 percent. The pattern of selection of *B. intermedia* and *B. pratensis* parasitized pupae and healthy pupae agrees with the data from the previous study (table 4-17). Wounded or injured pupae were preferred (χ^2, $P<0.005$) by the vertebrates (open category).

One area where predation information has been lacking is the 24-hour periodicity of predation. In an attempt to determine when the predation recorded in the three studies was occurring, the vertebrate boxes were examined for 3 days at 8-hour intervals (table 4-19). With a predator community as diverse and complex as that of the gypsy moth, it is not surprising that predation occurs 24 hours a day. The data in table 4-19 represent the periodicity of the ground inhabiting segment of the predator complex; however, all predators did not have access to the

pupae—for example, birds were excluded. Small-mammal predation (indicated in the open boxes) was highest from midnight to 8 a.m. and lowest from 8 a.m. to 4 p.m. The increase in percent eaten across all categories in the open boxes can be attributed to larger invertebrates and the smaller vertebrates (that is, *Sorex* spp., young *B. brevicauda,* and young *P. leucopus*). Had adult *B. brevicauda*—the largest and most abundant shrew on the study area and too large to get in the open boxes—been allowed access to these open boxes, the predation for all time periods would have been significantly higher.

Important and appropriate to this discussion of predator impact are some comments pertaining to the hypothesis As *P. leucopus* density increases through management, the rate of predation of gypsy moth pupae will also increase. In 1976, when pupae were presented to populations of managed *P. leucopus* (site 1), which had a density approximately two times that of the unmanaged mouse population (site 2), no significant difference in gypsy moth predation occurred. Conversely, in 1977 *P. leucopus* densities were similar, yet predation of pupae in the litter in the managed population was significantly greater.

The problem is that, although the hypothesis is a reasonable one, changing conditions (especially food resources) in the natural state make it extremely difficult to test and demonstrate. Hinde (1958) has proposed that an animal, while maturing, "learns to take those foods which its repertoire of behavior patterns and structure permit to exploit most efficiently," indicating that other variables actually outweigh the number of predators in terms of predicting predator effect. This is not to say that management is not feasible and should not be considered in attempts to control gypsy moth populations. However, without a more complete understanding of the hardwood ecosystem, predatory impact as a result of management will remain unpredictable.

These studies have confirmed some earlier beliefs about gypsy moth predators, disagreed with others, and given new insight into the importance of predation. Above all, they have emphasized the need

and direction of future research if managing an ecosystem as complex as a hardwood forest to control gypsy moth populations is to become a reality.

Observations on the greater susceptiblity of parasitized pupae to attack by all these predators indicate the need for further investigations. Perhaps most surprising was the abundance of activity of the invertebrates as they scavenged the parasitized pupae and attacked healthy pupae. High parasite mortality related either directly or indirectly to vertebrate and invertebrate predatory behavior may be a significant factor contributing to the ineffectiveness of parasites in this country (Reardon 1976, Campbell and Sloan 1977a). An appreciation of the complexity of this predator/prey system is gained when one remembers that these studies focused only on one life stage (the pupa), in one location (the litter), on one site (a forest).

Management Implications

Buckner (1966), in discussing the role of predators in the biological control of forest insects, stated "Particular emphasis should be placed on the factors governing the abundance of the various predators so that advantage may be gained from the large groups of potentially important predators whose numbers are not related closely to the abundance of the prey." Human activity and land use practices often affect the abundance and distribution of both insects and their predators. Because human activity and land use practices can have tremendous effect on species that are important predators of forest pest insects, it seems appropriate to make a few comments about the concept of managing these predators.

"The general needs of all animals are much alike in that each must have a sufficient quantity and variety of food and protective cover to meet its physiological needs for maintenance, growth and reproduction throughout its life" (Trippensee 1948). In all habitats there is a limitation on the number of animals of each species that an area can maintain or support; this is called the carrying capacity. The population of each species adjusts to the carrying capacity for each area. Every time a significant habitat change occurs in an

area, the animal populations, given proper time, readjust to the new carrying capacity. Depending on the actual change that took place, wildlife number and diversity can increase, decrease, or remain the same. Generally, if food and cover are increased, wildlife populations increase. However, each species does have certain limits beyond which its populations cannot increase. These limits are often referred to as tolerance density, a point where intraspecific competition and crowding take place and become the population's controlling mechanism.

Forest managers wishing to employ predator management for insect control can either increase native predator populations or release exotic predator species, or do both. The release of exotic predators is not and should not be an attempt to release a large number of predators available for immediate control—this will not work. Rather it should be an attempt to establish a breeding population of a particular predator where it previously did not exist, such as the case with *C. sycophanta*, and should be the case if the exotic pentatomid, *Dinorhynchus dybowskyi*, is released from quarantine.

The introduction of the masked shrew from mainland Canada into Newfoundland to control larch sawfly is one example of a successful attempt to establish a breeding population of a vertebrate predator to control a forest pest insect.

The idea of managing a particular predator or predators to combat a forest pest insect is not new. Buckner (1966) described a successful attempt by Hamilton and Cook in the 1930's to protect a plantation of larch in New York State from an outbreak of the larch sawfly. They supplied nest boxes and brush piles that provided a significant habitat change that resulted in an abundance of deer mice, redback voles, and masked shrews. Each of these mammals has a relatively high insectivorous diet, and the increase in their numbers led to a reduction in the number of sawflies in the managed plantations. In the unmanaged control areas, sawflies remained at outbreak levels for years.

Providing and/or improving food and cover for wildlife can increase the density of wildlife.

Populations of birds and mammals can often be increased simply by increasing the food supply. Feeding has the advantage of being easily turned on and off and, if properly manipulated, could change a species from eating the feed back to a natural diet, including insects, at the will of the manager. However, feeding has the disadvantage of being expensive, nonselective, and effective for only a short term.

A more reasonable way to increase population densities of useful vertebrates is through silvicultural habitat manipulation—encouraging plant species that have the capacity of supplying both food and cover. To manage a species through habitat manipulation, it is necessary to understand the habitat requirements for each species and whether these requirements can be manipulated in an economically and ecologically feasible manner to achieve the management objectives. In each case, it must be determined if the habitat can be manipulated in a way to increase the abundance of the vertebrates that eat gypsy moths.

Fortunately, many species of birds and small mammals respond favorably to relatively simple habitat changes. Creating dense ground cover, one of the most important requirements of forest-dwelling small mammals, can effectively increase small-mammal populations. Its importance for large populations of small mammals cannot be over-emphasized.

Populations of hole-nesting birds have been successfully increased by providing them with nest boxes. In parts of the Soviet Union, nest boxes have been used to increase bird numbers in an integrated management system for a complex of forest-defoliating insects, including the gypsy moth.

What can forest managers and homeowners do to help increase, maintain, and create a more diverse predator community? Homeowners, unfortunately, often seem compelled to clean up their property and turn naturally brushy areas into lawns, thereby removing the food and cover needed by birds and mammals. Foresters also are often involved in similar large-scale cleaning operations, which led to the elimination of the brush competing with the trees. These cleaning operations often drastically reduce

both vertebrate species diversity and the total number of birds and small mammals in the area. The elimination of brush removes the cover necessary for bird species, which occur from the ground to the lower canopy, and eliminates much of the cover necessary for small mammals.

Few people will manage their land strictly for small mammals and birds, but many foresters and home-owners unknowingly turn good wildlife habitat into unsuitable habitat simply by removing the brush. By understanding the requirements of these predators, one can actually manage for them by not destroying their habitat, or at least by trying to minimize the impact of human activities on the habitats of these animals.

It appears that the feasibility of managing predators decreases as the complexity of the system increases. Whittaker (1970) stated that mesic, temperate, deciduous forests are probably richest in species diversity of temperate forests of the world. The farther one gets from an agromonoculture system the more difficult it will be to develop an economically and biologically feasible management scheme that will be effective against a target insect, and there has been no known successful attempt to manage forest-pest predators in a deciduous hardwood forest to reduce insect damage.

Reexamining Our Wildlife Perceptions

The factors of predator potential, predator impact, alternative foods, prey and predator density, and food availability remind us that we are dealing with some of nature's normal, effective, and continually operating mechanisms, which are subject to change at any time.

Just as important as understanding the effects of these variables are the perceptions of wildlife that most people develop throughout their lives. Predator connotes large carnivores, while small animals such as mice, shrews, warblers, robins and even insects are seldom thought of as predators. A chipmunk is thought of with an acorn in its mouth rather than an insect pupa; a shrew, searching for worms rather than for gypsy moth caterpillars; or an ant, carrying away crumbs rather than gypsy moth eggs.

These perceptions can be difficult to change and often prevent us from seeing what is really happening. Campbell (1975) reported that shrews seldom came in contact with gypsy moths in the natural state. It has now been learned that shrews not only come in contact with gypsy moths but that they often eat pupae in significant amounts.

It took half a century from the time Forbush and Fernald (1896) discussed bird predation until mammals were mentioned as an important predator of the gypsy moth. Another 25 years passed before researchers began to quantify the predatory potential and impact of mammals on sparse gypsy moth populations. It should no longer be assumed that other groups of animals are insignificant as preda-tors of forest insects. Given certain circumstances or conditions, they may be important. They are all members of the predator community, and within that context they and their role in gypsy moth pop-ulation dynamics have been discussed. It is the collective impact from this complex predator com-munity that we should try to understand and utilize in future management programs. To successfully accomplish this, we must start by reexamining and adjusting our wildlife perceptions.

Conclusion

A principal objective throughout the gypsy moth research and development program has been to develop an understanding of the role and importance of predation in the population dynamics of the gypsy moth. Over the last few years a great deal has been learned about the gypsy moth predator complex and about several characteristics that make predators a major suppressive force in the population dynamics of the insect. However, further information is needed before knowledge of gypsy moth predator/prey theory can be successfully integrated with biologically and economically feasible practice so that forest managers can use predators in an effective and practical control program.

Particular emphasis in future studies should be placed on understanding the foods and feeding habits of the predators so that they can be used effectively in

an integrated pest management program. Also, because predator impact is so closely associated with alternative foods, a better understanding is needed of annual food production, variety, quality, and variability within northeastern hardwood forests.

The gypsy moth predator/prey system is complex, being composed of many predator species that eat a variety of foods. These predators are all opportunistic and select food largely as a function of availability and abundance. Although a preferred food may be eaten in great quantity, these predators attempt to vary their diets and are always testing other potential food sources; in this process gypsy moths are eaten by many predators. It is the collective impact of these predators that is important in maintaining certain sparse gypsy moth populations.

If the gypsy moth predator system were simple, one or a few predators dependent upon a particular pest insect for food, the impact and precise role of predation would be easier to predict and determine. On the other hand, the complexity of the gypsy moth predator community may actually give greater year-to-year stability and may be a major reason why some gypsy moth populations remain sparse and relatively stable for several years.

It is hoped that the information presented in this section has increased the awareness of the value and importance of predation in maintaining population stability of forest insect pests. It is known that predation is a regulatory factor in certain gypsy moth populations and in populations of some other forest pest insects. However, it must also be realized that predation is only one of the forces suppressing insect population growth in a forest ecosystem. Although at times predators may regulate gypsy moth populations, they cannot regulate them forever and they do not regulate them alone.

Predators have been and will continue to be important in the population dynamics of the gypsy moth. Gypsy moth populations in this country usually appear to be at either harmless or outbreak levels. One of the keys to the harmless level is the naturally occurring predation by the predator community. Because the best way to cope with a problem is to prevent it, it would be wise to include predators in future attempts at managing the gypsy moth.

As more is learned about predation and other processes within the forest ecosystem, an appreciation must grow of the importance of slowly and patiently gathering the facts from which conclusions are drawn. Objectivity must be maintained in order to put early preconceptions in their proper perspective and gather scientifically acceptable evidence from which new research goals are made and future pest management programs are developed. The success of future gypsy moth managment systems lies in our ability to appreciate, understand, and wisely use all the natural suppressive mortality factors operating within the forest ecosystem.

Natural Disease Within Dense Gypsy Moth Populations
John D. Podgwaite

Introduction

The gypsy moth is host to a variety of naturally occurring pathogens, parasites, and predators. Since the early 1900's, the nucleopolyhedrosis virus (NPV) of the insect has received the attention of many investigators and is generally considered to be the primary natural regulator in dense, North American gypsy moth populations (Reiff 1911, Glaser and Chapman 1913, Doane 1970a). *Bacillus thuringiensis* has also been widely studied because of its potential for use in control programs (Lewis and Connola 1966, Yendol et al. 1973, Lewis et al. 1974). Other pathogens have received less attention either because they are considered insignificant in natural population fluctuations or because they possess properties that contraindicate their candidacy as microbial control agents. These include the cytoplasmic polyhedrosis virus (CPV) of the insect (Magnoler 1970, 1974); a variety of bacteria, including *Streptococcus faecalis* (Cosenza and Lewis 1965, Doane and Redys 1970) and *Serratia marcescens* (Podgwaite and Cosenza 1976a,b); the fungi *Beauvaria bassiana* and *Paecilomyces farinosus* (Majchrowicz and Yendol 1973); and several species of microsporidia (Weiser 1961).

Additionally, little notice has been given the socalled "physiological" diseases of the gypsy moth, although their potential importance in dense populations has been indicated (Campbell and Podgwaite 1971).

Entomophagous parasites of the gypsy moth have been widely studied because of their obvious potential as biological control agents; however, it is generally assumed that these parasitoids are naturally regulatory only in sparse populations and play a lesser role after such populations become dense (Reardon 1976).

Vertebrate predators, including the white-footed mouse, *Peromyscus leucopus,* and several bird species also are major regulators in sparse populations, but again appear to have little impact when populations become dense (Campbell and Sloan 1977*b*).

Few studies have been directed toward understanding how the effects of all these mortality-causing agents integrate to bring about the natural regulation of dense gypsy moth populations. Such information is essential in determining when and how to manipulate the environment to maximize these effects.

Toward this goal, Campbell and Podgwaite (1971) compared the nature of disease in sparse and dense populations and found significant differences in both the kinds and numbers of disease agents operating in these two population types. Furthermore, the variation in disease found within the dense populations studied led to the conclusion that, in order to understand fully the disease complex of the insect, it must be studied across a number of years within a series of populations from different geographical areas. Thus, a study was designed to determine the components of disease operating in dense gypsy moth populations and to relate the variation in disease incidence to both biological and physical variation in the environment. The following discusses the nature and incidence of mortality-causing agents operating in these populations over a 2-year period.

Methods

Field

Sites ranging from 2 to 4 ha in area and supporting moderately dense to dense gypsy moth populations were established across six geographically distinct study areas in the Northeastern United States (fig. 4-34). Although study areas were selected so as to represent a wide range of environmental conditions, sites within a given area were chosen on the basis of their similarity with respect to host species composition, soil moisture, aspect, and egg-mass density. Within each site, five 0.25-ha plots were established for collecting gypsy moth life-table data. Ten trees, located on the perimeter of each plot and representing the dominant host species in the site, were selected for sampling diseased insects. Each tree was sampled by collecting dead and/or moribund larvae from the bole to a height of 2 m above the base and from three 0.25-m² collecting traps positioned so as to catch diseased insects falling from the crown. Certain sites in areas A, B, and F were selected for studying early first- through third-instar larval mortality. Collections in these sites commenced at the

Area	Location	1972 sites	1973 sites
A	Harwich, Mass.	4	2
B	Ludlow, Mass.	6	2
C	Whitehall, N.Y.	4	3
D	Cobleskill, N.Y.	2	2
E	Clinton, N.J.	4	3
F	New Lisbon, N.J.	3	3
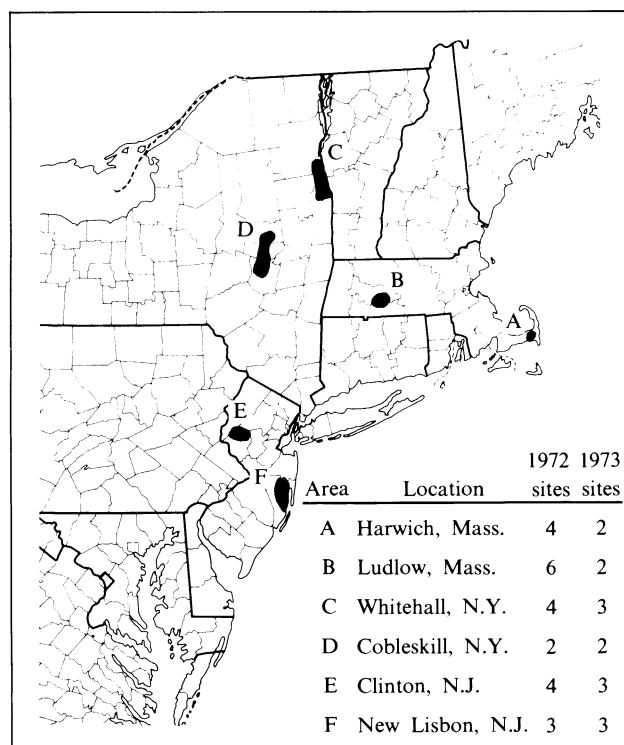
Figure 4-34.—*Gypsy moth study areas in the Northeastern United States.*

time of egg hatch. Collections in all other sites were initiated on or about June 1. All sampling points were visited twice daily until adult emergence. Every diseased gypsy moth larva or pupa found was placed in a sterile container, labeled, refrigerated, and forwarded to the laboratory for examination.

Laboratory

Upon delivery to the laboratory, each diseased insect was coded and examined in accordance with a scheme outlined in figure 4-35. Data taken at various stages in the exmination were entered on a precoded form that facilitated computer processing and analysis.

External Examination

With the aid of a dissecting microscope, insects were examined for gross pathologies, including

1. Coding
2. External examination
   - Gross pathology — Culture — Identify
     - Predator wound ——————— x
     - Parasite or wound ——————— x
     - Teratology–neoplasm
     - Fungi ——————— x
3. Surface sterilization
4. Dissection
5. Internal examination
   - Gross pathology
     - Parasite ——————— x
     - Nematode ——————— x
6. Triturate
7. Microscopic examination
   - NPV
   - Bacteria ——————— x ——— x
   - Fungi ——————— x ——— x
   - Protozoa
   - Other microorganisms
8. Diagnosis
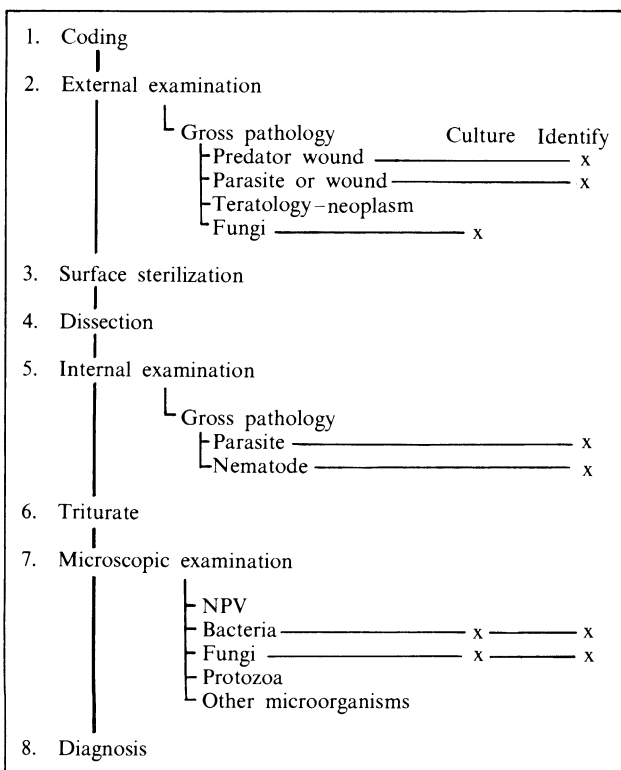
Figure 4-35.— *Protocol for the examination of diseased gypsy moth larvae and pupae.*

neoplasms, teratologies, evidence of parasitism, or predation and fungal infections. Wounds inflicted by vertebrate predators or emerging parasites were identified according to the keys described by Campbell (1963a). Immature stages of entomophagous parasites were preserved using a modification of a fixative developed by Hall et al. (1933) and later identified according to standard entomological keys. Insects supporting fungal growth were cultured by excising portions of the integument and plating on Sabouraud Dextrose Agar (SAB) incubated at 28° C for 7 days.

Internal Examination

Insects were individually surface treated for 5 minutes by agitation in an aqueous solution of 0.2 percent Hyamine 10–X containing 0.01 percent Trition X–100. Insects so treated were rinsed repeatedly in sterile distilled water to remove all traces of sterilant and then aseptically dissected. With the aid of a dissecting microscope, gross pathologies internal to the insect were noted. Any entomophagous parasites or nematodes were collected, fixed as above, and identified.

Microscopic Examination

Dissected cadavers were triturated in 1 ml of sterile distilled water and a loopful (5 mm) of the resulting suspension smeared evenly over 2 cm^2 on a glass slide, fixed, and stained with dilute Giemsa for 5 minutes. Twenty-five random fields per stained smear were examined at 1000× under oil immersion for microbial agents and/or histological and cytological evidence of disease. The numbers and types of microbial agents appearing were quantified on a per field basis. Additionally, wet mounts were prepared from suspensions and examined using phase contrast microscopy.

Isolation and Identification of Microorganisms

Bacteria were isolated from triturated cadaver suspensions showing 10 or more morphologically similar cells per field in stained smears. Isolations were accomplished by streaking a loopful of suspension on

trypticase soy agar (TSA) and then incubating aerobically at 30° C for 48 hours. At the conclusion of the incubation period, representative colonies were picked and identified using standard microbiological techniques. Identification of members of the Enterbacteriaceae were facilitated by using API diagnostic strips (Anyltab®).

Fungi were isolated when fungal spores or hyphal fragments appeared in numbers greater than 10 per field in stained smears. Isolations were made on SAB incubated at 28° C for 7 days. Isolates were grouped according to cultural characteristics and representatives of each group were identified using standard mycological methods.

No attempt was made to isolate and identify other microorganisms quantified in the microscopic examination.

Assigning Causes of Death

Each insect was assigned to one of the following cause-of-death categories: Predation, parasitization, nucleopolyhedrosis, bacteriosis, mycosis, physiological and undetermined. The criteria used for these categorizations are shown in table 4–22. The scheme had as its basis a priority ranking of known diseases and agents over those conditions and microorganisms

that had not heretofore been shown to unequivocally cause disease in the insect. The volume of diseased insects and microbial isolates precluded the identification of all infectious agents; therefore, diagnoses were made on the basis of gross pathology, microscopic examinations, and the identification of known gypsy moth pathogens. The scheme was not without pitfalls—for example, the categorization of an insect harboring two or more known pathogens. When the pathological data did not allow an unequivocal diagnosis, cause of death was labeled undetermined.

Results

In 1972, each of 28,542 diseased insects collected from 23 sites was examined and assigned to one of the cause-of-death categories listed in table 4–22. In 1973, 29,359 specimens from 16 of the 23 sites sampled in 1972 were similarly treated. Distributions of cause of death categories by year, area, and site are shown in tables 4–23 to 4–29. Trends across larval instars are seen in figure 4–36.

Since sampling techniques were biased toward collecting insects killed by parasites and pathogens, predation accounted for only a small percentage of the measured mortality in all sites: 0.4 (site range=0.0–2.7) percent in 1972, and 0.6 (0.0–2.4) percent in 1973.

Table 4–22.—*Protocol for assigning cause of death to diseased gypsy moth larvae and pupae*

Cause of death category	Criteria
1. Predation	Evidence of vertebrate or invertebrate predation alone or in combination with any other agent or condition except NPV.
2. Parasitized	Evidence of the parasites *Apanteles melanocelus, Parasetigena agilis* (=silvestris), *Compsilura concinnata,* or *Blepharipa pratensis* (=*scutellata*) alone or in combination with any other agent or condition except NPV and predation.
3. Nucleopolyhedrosis (NPV)	Polyhedral inclusion bodies of the nucleopolyhedrosis virus (Baculovirus) occurring in any numbers or in combination with any other agent or condition.
4. Bacteriosis	One or more morphologically distinct bacterial types occurring >10 per microscopic field in a standard smear either alone or in combination with any agent or condition except NPV, evidence of parasitism or predation or the fungi listed below.
5. Mycosis	Evidence of either *Paecilomyces farinosus, Beauvaria bassiana,* or *Aspergillus flavus* alone or in combination with any agent or condition except NPV and evidence of predation or parasitism.
6. Physiological	No evidence of infectious agents or overt cause of disease.
7. Undetermined	Any other than 1–6 above.

Table 4–23.—*Causes of gypsy moth mortality in all study areas, 1972–73*

Area	Year	Number of insects examined	Percent of total						
			Predators	Parasites	NPV	Bacteria	Mycosis	Physiological	Undetermined
A	1972	7,702	0.2	0.3	4.2	10.9	0.1	43.6	40.6
	1973	6,940	1.1	3.4	28.0	4.6	.1	42.9	19.6
B	1972	7,131	.4	2.3	20.3	14.1	.1	29.9	32.6
	1973	2,412	1.3	4.3	43.0	7.9	.3	34.9	8.1
C	1972	3.864	.1	2.4	34.8	26.3	.6	22.4	13.1
	1973	961	.8	13.3	29.1	10.4	1.9	24.3	19.9
D	1972	4,094	.7	4.6	19.8	11.7	0	12.5	50.6
	1973	1,801	.8	15.3	46.4	12.2	.2	13.9	11.0
E	1972	2.969	1.1	5.7	14.4	50.4	.2	18.1	10.0
	1973	7,303	.1	3.1	48.3	18.6	.5	16.3	12.9
F	1972	2,782	.1	0	19.8	11.8	0	62.4	5.6
	1973	9,942	.5	.3	31.5	8.0	.3	50.0	9.1
Total areas	1972	28,542	.4	2.2	17.2	18.4	.2	32.0	29.4
	1973	29,359	.6	3.4	36.6	10.2	.4	35.6	12.9

Table 4–24.—*Causes of gypsy moth mortality in sites within area A, 1972–73*

Site	Year	Number of insects examined	Percent of total						
			Predators	Parasites	NPV	Bacteria	Mycosis	Physiological	Undetermined
1	1972	2,396	0.1	0.5	3.4	7.3	0.1	41.0	47.4
	1973	2,486	.9	4.9	34.8	4.3	.2	36.2	18.9
3	1972	1,331	.1	.1	6.9	14.4	.2	69.8	8.4
	1973	4,451	1.1	2.6	24.4	4.8	.1	46.8	19.9
5	1972	1,346	.7	.6	4.1	4.5	.1	38.8	51.1
	1973	—	—	—	—	—	—	—	—
6	1972	2,629	.2	.1	3.6	15.9	0	34.7	45.4
	1973	—	—	—	—	—	—	—	—

Table 4-25.—*Causes of gypsy moth mortality in sites within area B, 1972-73*

Site	Year	Number of insects examined	Percent of total						
			Predators	Parasites	NPV	Bacteria	Mycosis	Physiological	Undetermined
1	1972	445	0.3	5.6	35.6	21.3	0.2	27.6	9.6
	1973	—	—	—	—	—	—	—	—
3	1972	1,088	.4	2.4	17.8	10.1	.1	14.3	54.7
	1973	—	—	—	—	—	—	—	—
4	1972	1,731	.4	2.3	19.7	13.6	.1	13.7	50.1
	1973		—	—	—	—	—	—	
5	1972	2,005	.4	2.2	15.8	14.1	.1	32.3	35.0
	1973	828	1.8	5.4	35.7	8.6	.3	37.6	10.4
6	1972	1,175	.5	.4	30.6	16.5	.1	45.8	6.0
	1973	1,042	.3	4.3	56.2	6.5	.1	26.5	5.9
8	1972	688	.3	3.6	12.3	13.5	0	63.1	7.1
	1973	540	2.4	2.6	28.9	9.8	.3	47.0	8.9

Table 4-26.—*Causes of gypsy moth mortality in sites within area C, 1972-73*

Site	Year	Number of insects examined	Percent of total						
			Predators	Parasites	NPV	Bacteria	Mycosis	Physiological	Undetermined
1	1972	1,803	0.1	1.7	35.4	30.6	0.6	25.4	6.0
	1973	271	.3	17.7	20.3	11.8	2.9	27.3	19.2
2	1972	521	0	1.9	31.6	27.6	.8	30.5	7.4
	1973	243	1.2	13.6	31.3	10.3	1.2	25.5	16.8
3	1972	256	.8	9.8	12.9	21.1	1.6	32.4	21.4
	1973	447	.9	10.5	33.3	9.6	1.8	21.9	21.9
4	1972	1,284	.1	2.3	39.7	20.7	.3	12.9	23.8
	1973	—	—	—	—	—	—	—	—

Table 4-27.—*Causes of gypsy moth mortality in sites within area D, 1972-73*

Site	Year	Number of insects examined	Percent of total						
			Predators	Parasites	NPV	Bacteria	Mycosis	Physiological	Undetermined
2	1972	2,436	0.7	3.1	22.1	11.4	0	11.7	50.8
	1973	692	.6	17.6	40.3	10.3	.4	15.6	15.1
7	1972	1,658	.6	6.7	16.4	12.1	.1	13.8	50.3
	1973	1,106	.9	13.8	50.3	13.4	.1	12.7	8.4

Table 4-28.—*Causes of gypsy moth mortality in sites within area E, 1972-73*

Site	Year	Number of insects examined	Percent of total						
			Predators	Parasites	NPV	Bacteria	Mycosis	Physiological	Undetermined
1	1972	1,254	0.2	1.6	9.3	66.1	0.2	12.4	9.9
	1973	549	.3	8.0	22.6	23.3	1.4	20.6	23.4
2	1972	561	1.4	5.1	30.6	30.1	.1	22.8	9.6
	1973	—	—	—	—	—	—	—	—
4	1972	518	2.7	16.2	5.0	46.9	.2	18.7	10.2
	1973	1,003	.1	1.2	20.2	6.9	.9	42.3	28.1
9	1972	636	1.3	5.6	17.6	40.4	.1	24.5	10.4
	1973	—	—	—	—	—	—	—	—
10	1972	—	—	—	—	—	—	—	—
	1973	5,748	0	3.0	55.6	20.1	.4	11.4	9.3

Table 4-29.—*Causes of gypsy moth mortality in sites within area F, 1972-73*

Site	Year	Number of insects examined	Percent of total						
			Predators	Parasites	NPV	Bacteria	Mycosis	Physiological	Undetermined
4	1972	294	0.3	0	12.2	9.8	0	72.4	5.1
	1973	4,712	.4	.2	22.5	9.8	.4	56.3	10.7
5	1972	1,701	.1	0	18.5	9.6	0	65.8	5.8
	1973	2,343	.4	.6	46.4	4.8	.3	40.3	7.1
6	1972	787	.1	0	25.5	17.3	0	51.4	5.6
	1973	2,885	.7	.3	34.1	8.7	.1	47.8	8.2
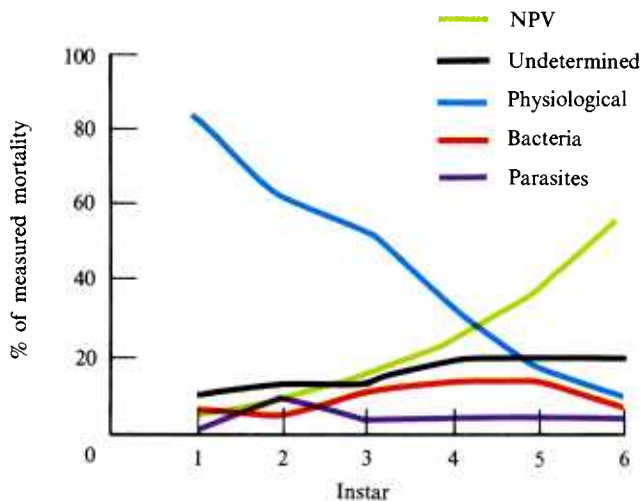
Figure 4–36.—*Major cause of mortality across larval instars in areas* A–F, *1972–73.*

This mortality was attributed to the activity of *Calasoma* spp. and to the vertebrate *Peromyscus leucopus* and did not differ among larval instars. Although other vertebrate and invertebrate predators were present in the study sites, their involvement could not be unequivocally defined.

Parasitoids were responsible for 2.2 (0.0–16.2) percent of the total measured mortality in 1972 and 3.4 (0.2–17.7) percent in 1973. When parasitism was relatively high, it was due to either *Compsilura concinnata* (area E, site 4, 1972) or *Blepharipa pratensis* (areas C and D, 1973). Other species contributing to the overall mortality caused by parasitoids were *Apanteles melanoscelus* and *Parasetigena silvestris*. Evidence of parasitism in first-instar larvae was virtually nonexistent but increased to a maximum in the second instar, due almost entirely to *A. melanoscelus*. Parasitism across third through sixth instars was constant but slightly higher in 1973 than in 1972. This mortality was attributed to the aforementioned tachinid flies. Immature stages of members of the families Sarcophagidae and Phoridae were frequently recovered from diseased specimens but were not considered parasitic.

NPV accounted for 17.2 (3.4–39.7) percent of the measured mortality in 1972 and 36.6 (20.2–56.2) percent in 1973. As shown in figure 4–36, mortality from NPV increased across the instars from a low in first-instar larvae to a high in sixth-instar larvae. This relationship generally held for all sites except those in area A (1972) where virus activity peaked at a low level in fourth-instar insects and was somewhat reduced in fifth- and sixth-instar larvae.

Bacterial disease accounted for 18.4 (4.5–66.1) percent of the measured mortality in 1972 and 10.2 (4.3–23.3) percent in 1973. The incidence of bacterial disease was higher in those insects collected from area E in 1972 than in those collected from other areas. The major bacterial species involved were *Streptococcus faecalis*, *Serratia liquefaciens*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Bacterial diseases were higher in the fourth through fifth instar than in the first through third instars.

Only 0.2 (0.0–1.6) percent of the measured mortality in 1972 was attributed to mycoses. In 1973, it was slightly higher—0.4 (0.1–2.9) percent. The fungal pathogens involved were *Beauvaria bassiana*, *Paecilomyces farinosus*, and *Aspergillus flavus*. Fungal diseases were absent in first- through third-instar larvae in 1972 but not markedly different across all other instars in 1972 and 1973.

Physiological (noninfectious) disorders accounted for 32 (11.7–72.4) percent of the measured mortality in 1972, the highest of any category, and 35.6 (11.4–47.0) percent in 1973. In both 1972 and 1973, physiological diseases were higher in those insects collected from areas *A* and *F*. Mortality from physiological causes decreased across instars from a high in first-instar larvae to a low in sixth-instar larvae.

Diagnoses were categorized as undetermined in 29.4 (5.1–54.7) percent of the insects examined in 1972 and 12.9 (5.9–28.1) percent in 1973. The majority of specimens categorized as undetermined contained low levels (<10 per microscopic field) of one or more bacterial or fungal biotypes. In 1972 undetermined diagnoses were markedly higher in fourth- through

sixth-instar larvae than in first- through third-instar larvae. In 1973 this pattern was not evident.

Discussion

The wide variation in mortality caused by any one agent or complex of diseases from site to site within and among the areas studied precludes any fail-safe prediction as to what agents are going to predominate in any given dense gypsy moth population. However, the results do point to NPV and the complex of "physiological" diseases as those most likely to remove a significant portion of insects from dense populations.

Figure 4-36 probably represents what happens in the majority of dense populations that are in the decline phase. Data gathered in this study and a previous one (Campbell and Podgwaite 1971) suggest that the bulk of the mortality, exclusive of predation, occuring in the first through third instars is due to a complex of physiological disorders. Although these disorders have not yet been defined, they are undoubtedly related to factors in the insects' environment (weather, nutrition, etc.) and the genetic potential of the insect. The latter may program a late or early hatch and thus place the emerging larvae in a vulnerable position with respect to weather and food source. As physiological disease wanes in the third and fourth instars, it is "replaced" by an increasing incidence of NPV, which peaks at high levels in the fifth and sixth instars. This is not always the case, however, because in 1972 later stage larvae in dense populations on Cape Cod (area *A*) and in southern New Jersey (area *F*) did not succumb to NPV when stressed by lack of food but died of physiological causes undoubtedly related to nutritional deficiencies. A more detailed discussion of NPV epizootiology is presented in chapter 6.3.

Infectious diseases other than NPV appear to remove more later stage larvae from dense populations, but their incidence rarely approaches that of either NPV or "physiological" disease. These infectious diseases are caused primarily by the bacteria *Serratia marcescens*, *Serratia liquefaciens*, *Streptococcus faecalis*, *Pseudomonas* spp., and *Bacillus* spp. The collective mortality caused by these microorganisms usually does not exceed 15 percent on the average; however, there are at times "pockets" of bacterial disease that may account for up to 60 percent of the total mortality within a given area. A case in point is area *E* (northern New Jersey), where half the measured mortality in 1972 was caused by the pathogenic bacteria mentioned above, primarily *S. faecalis* and *Pseudomonas aeruginosa*.

S. faecalis has previously been implicated as endemic in dense gypsy moth populations (Cosenza and Lewis 1965, Podgwaite and Campbell 1972), and occasionally the disease caused by this microorganism reaches epizootic proportions (Doane 1970*b*). Although this bacterium may remove a significant number of larvae from a small area, its potential for causing widespread epizootics is probably limited by environmental factors.

Since fungi accounted for only 0.3 percent of the mortality recorded in this study, they are probably of little importance in overall population regulation. However, they may, when environmental conditions are optimal, remove a large proportion of the insects from a small area. The major fungal pathogens encountered in this study were *Beauvaria bassiana* and *Paecilomyces farinosus*, species previously reported to be operating in gypsy moth populations (Majchrowicz and Yendol 1973). *Conidiobolus coronatus* has also been reported as pathogenic for gypsy moth larvae (Hartman and Wasti 1974) but not in natural populations. It was not encountered in this study.

Other infectious agents, such as the cytoplasmic polyhedrosis virus that is regulatory in Japanese and European gypsy moth populations, and protozoans, which also contribute significantly to the decline of dense European gypsy moth populations, were not found in this study. In fact, these microorganisms

have never been reported as operative in North American gypsy moth populations.

Figure 4–36 indicates a secondary role for entomophagous parasites in dense larval populations. Undoubtedly, as is the case with the majority of the infectious agents mentioned above, parasites may occasionally remove a significant number of larvae from a given area (for example, areas *B* and *C* in 1972), but they do not appear to have the potential for causing widespread collapses of dense populations. A more important role for parasites within these dense populations may be as vectors of disease agents, particularly NPV. Analysis of data from this study reported elsewhere (Reardon and Podgwaite 1976) points toward this potential.

In this study the amount of measured predation was very low, less than 1 percent of the total over the 2-year period. Since many predators either consumed the whole insect, or removed it to areas that were not sampled, this figure is probably not indicative of their true impact on these populations. However, their limited numbers with respect to their dense host populations indicated that their impact was probably not great, particularly if alternate food sources were available. Although it was not measured in this study, predators may play a role in removing residual pupae after dense larval populations have been decimated by disease. Their collective activity at this time may impact on succeeding generations.

Future Studies

Results of this study have reiterated the major role of NPV in dense gypsy moth population declines. Certainly, a similar role for physiological disease cannot be discounted, particularly in dense populations when food is limited. Because these two disease categories are of paramount importance in the dynamics of dense populations, future studies should be directed toward an understanding of NPV epidemiology, in particular those environmental factors that may predispose populations to NPV epizootics; an understanding of the relationships between nutrition and disease; and an understanding of how the predisposition of the insect to both NPV and physiological disease is modified by the genetic potential of the gypsy moth itself.

Host Defoliation Effects Upon the Gypsy Moth
by William E. Wallner and Gerald S. Walton

Introduction

It is generally accepted that the basic qualitative nutritional requirements of insects vary little but that relative nutritional balance is of primary importance (Dadd 1973). The fact that the primary components of the foliage of most hosts are qualitatively similar led Beck and Reese (1975) to emphasize the role of secondary metabolites in insect/host plant interactions. Whereas host response to repeated gypsy moth defoliation has been documented (mortality and crown condition, Campbell and Valentine 1972; stand composition, (Campbell and Sloan 1977a) little is known as to how defoliation alters hosts as a nutritional source for gypsy moth. The quality of foliage as influenced by two or more drought years led Vasić (1950) and Benkevich (1964) to conclude that it was an important mechanism in the transition from latency to outbreaks in gypsy moth populations. This same relationship was described by White (1974) for outbreaks of certain lepidoptera that he believed were correlated with the abundance of foliar nitrogen. He theorized that trees stressed by random fluctuations in rainfall become nitrogen enriched and thus provide for increased survival of young larvae.

Edel'man (1963b) reported that the physiological condition of the gypsy moth was influenced by changes in the biochemical composition of its food. He further postulated that seasonal variation in nutritional composition was responsible for population outbreaks. Yolk quality and quantity were implicated by Capinera et al. (1977) to influence population quality. This difference in ovarian quality

has been inferred to be correlated with nutrition for *L. dispar* (Leonard 1970*a*) and western tent caterpillar (Wellington and Maelzer 1967). Indeed, Patočka and Cápek (1971) attributed alteration of host quality by defoliation to reduce *L. dispar* populations but no effort was made to document specific nutritional permutations.

The importance of nutrition upon the quality of individual gypsy moth larvae (dispersal and development rate) has been imputed (Leonard 1970*b*, Capinera and Barbosa 1977). Additionally, the type of host upon which gypsy moth fed was found to determine survival, developmental rate, and fecundity (Hough and Pimentel 1978). Mass reproduction of *L. dispar* was correlated with host nutrition by Kansu (1962) and Varga (1968), but specific components were not identified. Additionally, Merker (1964) reduced *L. dispar* populations by fertilization with carbonate of lime and ammonium sulfate. These studies suggest that host nutritional quality may have a profound effect upon the dynamics of *L. dispar* populations. Withholding of food to alter development rate, fecundity and dispersal was reported by Leonard (1970*a*); he believed that *L. dispar* is self-regulating because reduced nutrient quantity in the eggs imparted from the previous generation led to a prolonged first instar and a greater propensity to disperse.

Gypsy moth populations in Eastern North America are characteristically episodic with outbreaks seldom lasting longer than 3 years. Exogenous factors such as parasites, predators, and disease are normally implicated as regulating mechanisms of dense populations. In other instances, starvation is given as a contributing factor in population crashes. Under these conditions, larvae, after completely stripping the foliage from the trees, wander aimlessly in search of food, fail to mature, or, if they do, pupate prematurely. It is plausible that defoliation by *L. dispar* alters the composition of hosts making them nutritionally less suitable. Insects are known to alter forest growth and production (Mattson and Addey 1975); hence, it could be anticipated that a

defoliation/nutritional feedback exists. This was demonstrated by Haukioja and Niemela (1977), who, by mechanically damaging the foliage of birch, retarded the growth of *Operina autumnata*. The exact mechanism for disrupting normal development was unknown, but nutrient imbalance was suspect.

Methods

Should *L. dispar* limit itself through its host by modifying the composition of the foliage through defoliation, it could be an important factor in the population dynamics of this insect. Accordingly, studies were designed to compare the effect upon gypsy moth of feeding on two hosts undefoliated or artificially defoliated for 1 to 2 years in Killingworth, Conn. Eighteen black oak (*Quercus velutina*) and 18 gray birch (*Betula populifolia*) trees 6 to 8 m in height were defoliated for 1 or 2 years or left undefoliated. Each year larvae from three different geographic source populations were reared on each tree. The three source populations in 1976 were (A) Stroudsburg, Pa., (B) Harrisburg, Pa., and (C) Ludlow, Mass. In 1977 collections were taken from (D) Eastford, Conn., (E) Harrisburg, Pa., and (F) Milford, Pa. Five first-instar larvae were placed in 150 mm petri dishes with their sides vented with plastic screening. A 0.5-cm hole in the side of the dish permitted a twig to be inserted and the dish mounted on the tree with the foliage and larvae enclosed (fig. 4–37). Oaks and birches were infested on the same day with larvae of the same age by placing three dishes from each of the three source populations on every tree. Each dish contained five larvae; dishes were examined every other day; number and instars of live larvae were recorded, dead larvae were removed and their cause of deaths were noted.

Larval mortality, developmental time (from first instar to pupation), pupal weight within 24 hours of pupation, sex ratio, and morphological anomalies were criteria evaluated. Oaks were defoliated by cutting one-third of the leaves on each of three dates coincident with the majority of larvae achieving third,

fourth, and fifth instars. On the birches leaf cutting was impractical; every third leaf, then every second, and finally the remaining leaves were excised on the same schedule as for the oaks. Nine of the 18 trees of each species were defoliated in 1976. During 1977 those trees defoliated in 1976 were again defoliated, and six trees of each species were defoliated for the first time. Three trees of each species were left undefoliated. Since foliage was always available to larvae, even to those on trees being defoliated, variations in development were products of change in foliar content, not supply.

Figure 4–37.—*Petri dish cage with screen vented covers for constraining gypsy moth larvae on the foliage.*

Results

Artificial defoliation of both oak and birch significantly reduced pupal weights of six different *L. dispar* populations in the year of defoliation (tables 4–30 and 4–31; figs. 4–38, 4–39 and 4–40) in 1976 and 1977 Maksimović (1958a) and Hough and Pimentel (1978)

reported that pupal weight is correlated with fecundity. Hence, defoliation reduced the number of progeny per female and produced a diminution of population. A similar feedback mechanism was reported by Baltensweiler (1964) for the gray larch bud moth, *Zeiraphera griseana*, feeding on larch in Switzerland. He found that foliage produced the year
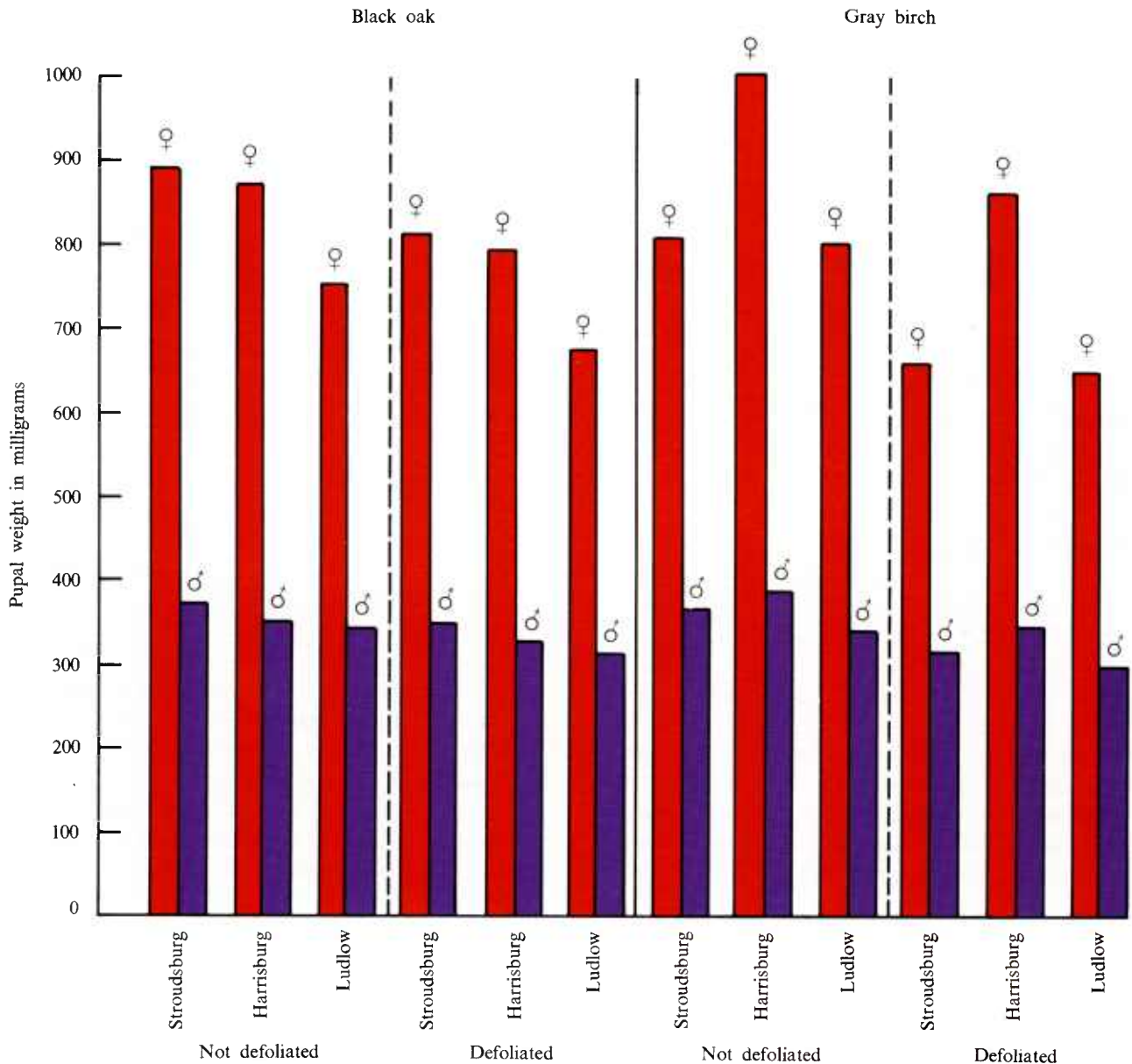
Figure 4–38.—*Reduced ANOVA model estimates of* L. dispar *pupal weights (mg). Larvae originating from eggs from Stroudsburg, and Harrisburg, Pa., and Ludlow, Mass., reared on the same undefoliated, and once-defoliated, black oak and gray birch trees in Killingworth, Conn., 1976.*

Table 4-30.—*Analysis of variance of gypsy moth pupal weights as influenced by defoliation, host, and geographic source (Killingworth, Conn., 1976).*

Source	Females		Males	
	Degrees of freedom	F	Degrees of freedom	F
Defoliation	1	18.18c	1	21.15c
Geographic source	2	13.17c	2	5.40b
Defoliation×tree species	1	4.21a	1	1.32NS
Geographic source×tree species	2	6.24c	2	4.82b
Time to pupation	1	11.31c	1	1.69NS
Model reduction	5	1.47NS	5	1.17NS
Full model error	256	(MSE=353.404)	235	(MSE=33.503)

Note: a=0.05 significance level; b=0.01 significance level; c=0.001 significance level.

Table 4-31.—*Analysis of variance of gypsy moth pupal weights as influenced by defoliation, host and geographic source (Killingworth, Conn., 1977)*

Source	Females		Males	
	Degrees of freedom	F	Degrees of freedom	F
Defoliation	2	5.13 b	2	13.43 c
Geographic source	2	3.84 a	2	.14 NS
Tree species	1	14.17 b	1	16.34 c
Defoliation×tree species	2	1.91 NS	2	3.77 a
Time to pupation	1	36.34 c	1	5.03 a
Model reduction	10	1.35 NS	10	.014 NS
Full model error	78	(MSE=0.02599)	200	(MSE=0.006606

Note: a=0.05 significance level; b=0.01 significance level; c= 0.001 significance level.

following defoliation was unfavorable as food for the larvae. The reproductive ability of individuals was reduced, and this tended to decrease the population.

Leonard's (1970b) laboratory results suggest that *L. dispar* can, through a change in nutrition, impose a selfregulating mechanism. Indeed, two successive defoliations resulted in significant reductions in pupal weight (table 4-31, figs. 4-39 and 4-40). There was a decrease in pupal weight in proportion to the number of years of defoliation on birch. However, on black oak the pupae were slightly larger on trees defoliated for 2 years than those defoliated only once. This increase, while not statistically significant, does suggest that differences in host nutritional resiliency exist and that for *L. dispar* black oak appears to be a more suitable host.

Differences among hosts were also evident in respect to developmental time. On undefoliated oak trees, larvae of both sexes developed 7.4±1.3 days faster than their counterparts on undefoliated birch. Development required 4.1±1.1 days and 2.5±1.0 days more on once- and twice-defoliated trees, respectively, than on undefoliated oak trees. Differences in developmental time due to defoliation of birch were not significantly different. By comparing developmental time (table 4-32, figs. 4-41 and 4-42) with pupal weight (table 4-31, figs. 4-39 and 4-40) it is apparent that larvae reared on undefoliated trees were larger yet required less time to develop than cohorts reared on trees defoliated 1 or 2 years. Alteration of the nutritional composition of the trees is believed responsible for these anomalies. Organic and inorganic
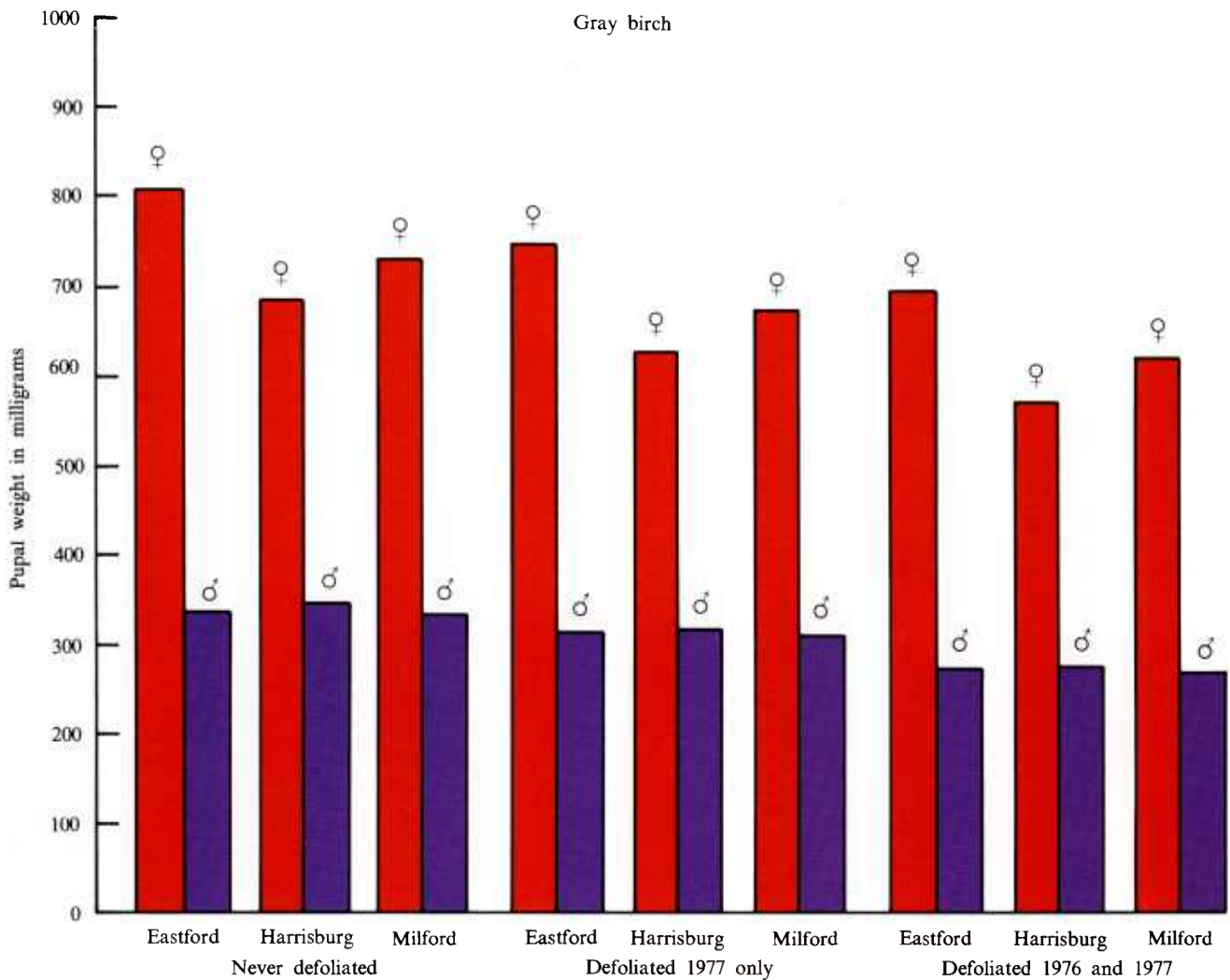
Figure 4–39.—*Reduced ANOVA model estimates of* L. dispar *pupal weights (mg). Larvae originating from eggs from Eastford, Conn., and Harrisburg and* *Milford, Pa., reared on the same undefoliated, once-defoliated, and twice-defoliated gray birch trees in Killingworth, Conn., 1977.*

constituent analysis of foliage from these study trees is continuing in an effort to correlate nutritional composition of the host with the anomalies observed in *L. dispar* development.

Various geographical source populations appear to differ from one another in developmental time and pupal weight even when reared on the same host. This suggests that there are subtle qualitative differences that may serve as discriminating criteria in characterizing *L. dispar* populations. It may also be

that the quality of the population is reflecting its immediate past history. Therefore, critical comparisons among populations should anticipate the prospect of nonconformity.

The biological significance of reduced pupal weights and extended developmental time has not been determined. However, defoliation, by extending developmental time, increases the opportunity for predation, parasitism, or other mortality factors. Reduced pupal weights, in addition to reducing the
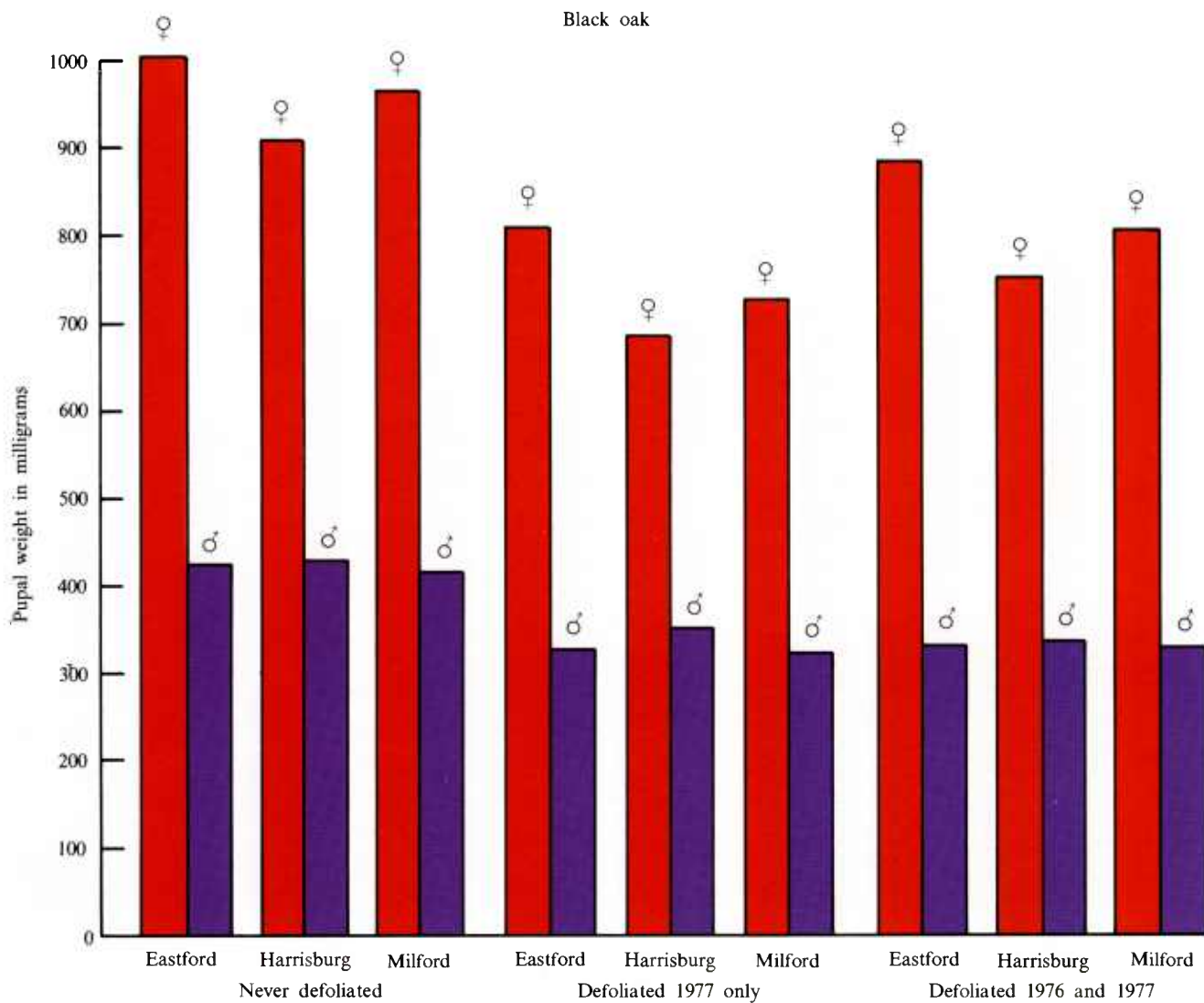
Black oak

Figure 4-40.—*Reduced ANOVA model estimates of* L. dispar *pupal weights (mg). Larvae originating from Eastford, Conn., and Harrisburg and Milford, Pa., reared on the same undefoliated, once-defoliated, and twice-defoliated black oak trees in Killingworth, Conn., 1977.*

Figure 4-42.—*Reduced ANOVA model estimates of* L. dispar *developmental time (days) from egg hatch to pupation. Larvae originating from Eastford, Conn., and Harrisburg and Milford, Pa., reared on the same undefoliated, once-defoliated, and twice-defoliated gray birch trees in Killingworth, Conn., 1977.* ▶
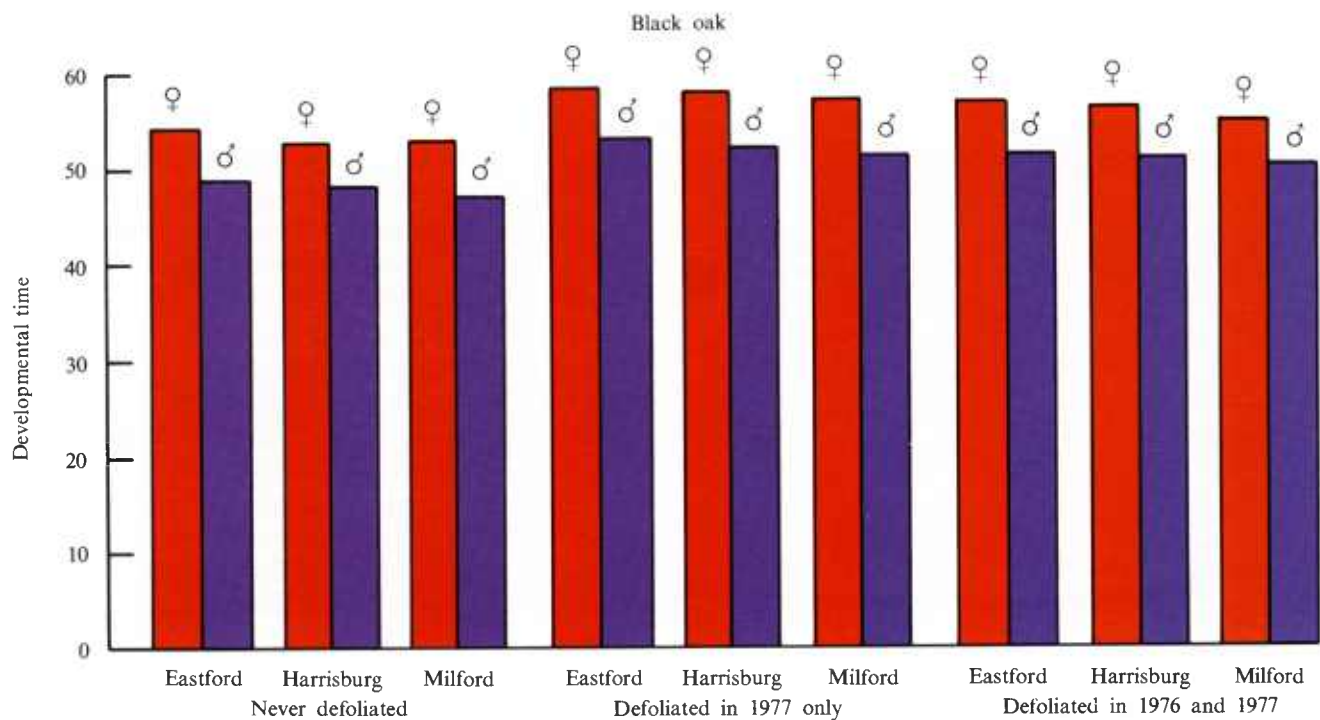
Figure 4-41.—*Reduced ANOVA model estimates of* L. dispar *developmental time* (days) *from egg hatch to pupation. Larvae originating from Eastford, Conn.,* *and Harrisburg and Milford, Pa., reared on the same undefoliated, once-defoliated, and twice-defoliated black oak trees in Killingworth, Conn., 1977.*
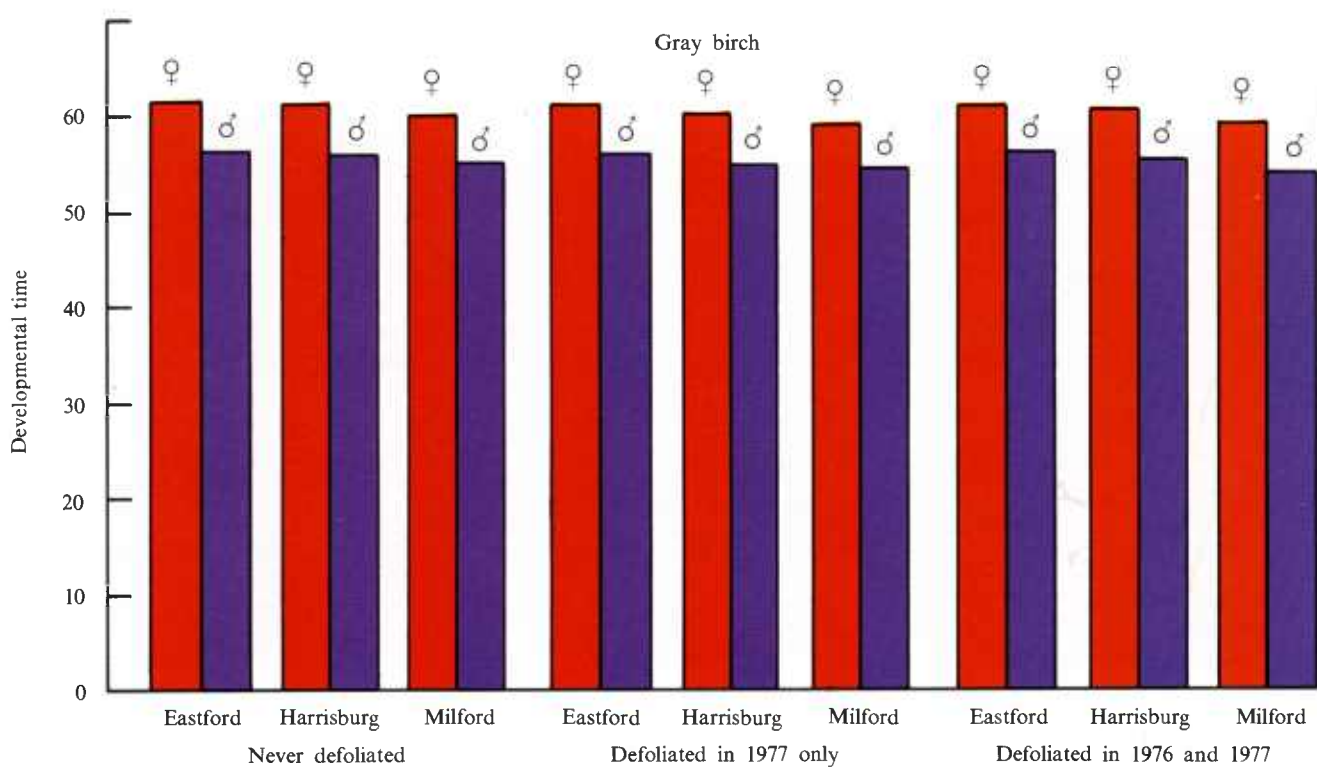
fecundity and vigor of the population, presumably are an expression of stress. One would therefore anticipate increased susceptibility to infectious disease or physiological debility. Examination of cadavers gave

no indication that any pathogen was expressing itself as a result of defoliation treatment or tree species (tables 4–33, 4–34, and 4–35). The major factors contributing to larval reduction were escape, loss through examination, dish cages being dislodged from trees by wind, and relocating larvae on the same tree (primarily first- and second-instar larvae due ostensibly to their small size). Those first instars that refused to initiate feeding eventually managed to escape from the dish cages where the twigs were inserted. The year-to-year consistency of losses to these factors among different source populations and tree species underlines the difficulty of constraining and observing larvae feeding on foliage in the field.

Host species effects upon mortality from third-instar larvae to adults of different geographic source populations were different. Mortality was consistently higher in both 1976 and 1977 on birch than oak with the exception of the Ludlow, Mass. (C) population in 1976 (table 4–36). These differences were not

Table 4–32.—*Analysis of variance of gypsy moth time of development as influenced by defoliation, host, and geographic source* (*Killingworth, Conn., 1977*)

Source	Degrees of freedom	F
Tree species	1	52.16 a
Geographic source	2	4.21 a
Defoliation treatment	2	2.33 NS
Sex	1	58.20 c
Tree species× defoliation treatment	2	4.67 b
Model reduction	27	.89 NS
Full model error	281	(MSE=28.334)

Note: a=0.05 significance level; b=0.01 significance level; c= 0.001 significance level.

Table 4–33.—*Gypsy moth survival and mortality by cause for three source populations reared on undefoliated gray birch and black oak* (*Killingworth, Conn., 1977*)

Population	Parasitism by Apanteles melanoscelus		NPV		Other		Survival		
	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Initial
First through fifth instar	3.7	10	1.1	3	65.2	174	30.0	80	267
Female sixth instar, prepupae and pupae	10.0	3	3.3	1	13.3	4	73.3	22	30
Male prepupae and pupae	0.0	0	0.0	0	8.0	4	92.0	46	50

Table 4–34.—*Gypsy moth survival and mortality by cause for three source populations reared on gray birch and black oak which were in the process of being defoliated* (*Killingworth, Conn., 1977*)

Population	Parasitism by Apanteles melanoscelus		NPV		Other		Survival		
	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Initial
First through fifth instar	1.9	10	1.7	9	71.7	387	24.8	134	540
Female sixth instar, prepupae and pupae	3.6	2	5.4	3	16.1	9	75.0	42	56
Male prepupae and pupae	0.0	0	0.0	0	5.1	4	94.9	74	78

significantly different hence data were pooled for oaks and birches and all geographic populations to compare treatment effects. Mortality was significantly different between defoliation treatments (table 4-37). Trees in the process of being defoliated caused significantly higher mortality to larvae feeding on them than those that remained undefoliated. Mortality increased progressively with defoliation.

Parasitism of larvae was primarily by *Apanteles melanoscelus*. Adults were observed gaining entry into the petri dish cages through the hole in which the twig was inserted. While parasitism did not exceed 5

Table 4-35.—*Gypsy moth survival and mortality by cause for three source populations reared on gray birch and black oak defoliated in 1976 and in the process of being defoliated for the second time (Killingworth, Conn., 1977)*

	Cause								
	Parasitism by *Apanteles melanoscelus*		NPV		Other		Survival		
Population	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Initial
First through fifth instar	4.0	30	3.1	23	70.6	526	22.3	166	745
Female sixth instar, prepupae and pupae	4.5	3	9.0	6	19.4	13	67.2	45	67
Male prepupae and pupae	0.0	0	0.0	0	11.1	11	88.9	88	99

Table 4-36.—*Host species effects upon mortality from third-instar larvae to adult eclosion for L. dispar by geographic population (Killingworth, Conn., 1976 and 1977)*

	Black oak		Gray birch	
Geographic population and year	Number of third-instar larvae	Percent mortality of third-instar through adult	Number of third-instar larvae	Percent mortality of third-instar through adult
1976				
Stroudsburg, Pa.	198	38.4	162	41.4
Harrisburg, Pa.	102	34.3	67	48.7
Ludlow, Mass.	195	54.9	95	49.2
1977				
Eastford, Conn.	101	35.6	95	49.5
Harrisburg, Pa.	114	47.4	105	39.3
Milford, Pa.	113	35.4	78	53.8

Table 4-37.—*Defoliation effects upon mortality from third-instar to adult eclosion for L. dispar from six geographic populations reared on oak and birch (Killingworth, Conn., 1976 and 1977)*

	1976		1977	
Treatment	Number of third-instar larvae	Percent mortality[a] of third-instar through adult	Number of third-instar larvae	Percent mortality[a] of third-instar through adult
Undefoliated	471	38.1	111	38.7
1 defoliation	469	52.0	119	42.2
2 defoliations	—	—	299	52.2

[a] All mortality rates in any one year are significantly different from one another. ($P<.05$) except the undefoliated vs. 1 defoliation in 1977.

percent in any year, it was consistently higher on black oak than on gray birch. Mortality by *A. melanoscelus* was considered minor, yet it demonstrated the persistent searching capability of this parasite.

Campbell and Podgwaite (1971) assessed the disease complex of *L. dispar* and reported that noninfectious disease or undetermined mortality accounted for more than 30 percent in dense populations. This was greater than any other mortality factor considered by them in their studies. It was impossible for them to differentiate between those larvae physiologically impaired and subsequently invaded by organisms, such as bacteria, and those originally infected by an organism. This situation is sufficiently important to the dynamics of *L. dispar* that studies are continuing to challenge with NPV, larvae emanating from eggs produced from these host-defoliation studies.

Sex ratios have received little but academic treatment in most *L. dispar* studies. Vasić (1950) suggested that factors such as food and temperature may cause shifts in sex ratios. Campbell (1967) reported that embryos and newly hatched larvae approximated a 50:50 ratio. However, he assessed only one (Glenville, N.Y.) population. Differential mortality of larvae and pupae was reported by Campbell (1963) to distort the sex ratio of gypsy moth. He found that disease and desiccation during late instars and prepupae were selective against females, whereas males were reduced by the attack of ichneumonids. The results of the present study agree with those of Campbell whereby female survival was consistently lower than that of males irrespective of geographic source population. This did not appear to be related to tree species or defoliation treatment, and it was not related to other factors such as disease or desiccation.

Differences observed in these studies suggest that sex ratios for populations may differ from one another. While it was not possible to ascertain the sex of larvae lost through handling or escape, trends observed indicate sex ratio distortions. In these studies pupal sex ratios differed significantly by source in 1976 (female to male, 57:43, 40:60, and 53:47 for Stroudsburg and Harrisburg, Pa., and Ludlow,

Mass). However, in 1977 the three geographic sources were not significantly different from one another but two were influenced by host species (Harrisburg, Pa., 43:57 on oak and 20:80 on birch, and Milford, Pa., 21:79 on oak and 42:58 on birch). There was no indication that defoliation or temperature differences due to caging affected sex ratios. Sex ratio distortions were also observed by Bell and Forrester (1977), who evaluated entire egg masses from certain wild populations and found male-to-female ratios of 60:40. They noted that sex ratio distortions favoring males were characterized by a population with a high incidence of NPV and parasitism.

While shifts in sex ratios from 50:50 appear to be innocuous, they may prove to be a measure of the quality of the population. Furthermore, defoliation estimates based largely on egg-mass density have been unpredictable. Since females consume three to four times as much food as males, sex ratio shifts would indeed influence anticipated defoliation.

Summary and Suggested Future Research

Artificial defoliation of black oak and gray birch is considered comparable to defoliation by natural dense *L. dispar* populations. It will alter normal developmental processes and cause progressive mortality and is believed to be a factor which contributes to the demise of outbreaks. Analysis of organic and inorganic constituents of foliage taken during this study is continuing and should provide insights into the nutritional requirements of *L. dispar*. However, several research areas that require investigation have been identified during the course of this study. The role of defoliation in producing qualitative changes in the remaining foliage is intriguing; after all, those insects feeding on trees being defoliated will suffer nutritional debility. Therefore, larval behavior in avoiding or selecting for defoliated foliage needs to be clarified.

It is assumed that reduced pupal weight and extended developmental time are expressive of a stressed insect. Are *L. dispar* progeny from defoliated trees more susceptible to disease than their cohorts reared on undefoliated trees? What behavioral

differences are evident as a result of being reared on hosts of suboptimal nutritional value? Could such information be used to assess more accurately wild populations and predict trends? Are there subtle differences among various geographic source populations that could be used to characterize and predict their dynamics?

The relationship between the phytophage *L. dispar* and its host continues to be poorly understood. Research initiated during this research program, some of which has been concluded, elucidates the complexity and importance of this interaction. Sufficient numbers of unanswered questions persist, making a more thorough assessment of this topic highly desirable.

Meteorological Influences

Introduction
William E. Wallner

Midway through the gypsy moth program, the need was recognized for predicting life-stage phenology and its relationship to host development. This was essential to suppression and trapping programs in newly infested regions where observations of life stages were impossible because of extremely sparse population levels.

Although a variety of reports is extant regarding the influence of meteorological phenomena on the survival and development of gypsy moth, no attempt has been made to review the world literature base and assimilate this information for constructing a predictive model.

Efforts within the gypsy moth program have resulted in capturing and utilizing available literature for preliminary model construction. The following discussion on the effect of meteorological phenomena on various life stages is based upon data derived from previous studies as well as those conducted within the gypsy moth program.

Egg Development and Diapause
Ronald L. Giese and Richard A. Casagrande

It is generally accepted that the egg stage of the gypsy moth undergoes three developmental phases

between oviposition and hatching: Early embryonic development, diapause, and a postdiapause incubation period. A great number of researchers have studied egg development, and over the years a general qualitative understanding has developed. Quantitative observations on developmental rates, hatching dates, mortality, etc., have varied considerably as measured by scientists around the world. These varied results have greatly complicated efforts to synthesize diverse experimental results into a unified model of egg development. A recent report by Giese and Cittadino (1977) includes an extensive review of literature pertaining to egg diapause in the gypsy moth.

Early Embryonic Development

Embryonic development begins immediately following oviposition and continues until an apparently developed larva exists within the egg. This development requires about a month in the field (Maksimović 1958b). In the lab, Pantyukhov (1962) found embryonic development to last 15 days at 25° C, while Leonard (1968b) found development completed during the fourth week of exposure to a temperature of 23.5° C. Development time prior to diapause was shown by Pantyukhov (1962) to be 81 days at 10° C, but, when held at 30° C, time for development was reduced to 13.5 days (fig. 4-43).

Upon completion of the early embryonic phase of development, a small proportion of gypsy moth eggs hatch without undergoing diapause. Forbush and Fernald (1896) found that 4 percent of a Massachusetts population hatched in this way. In field populations, the resulting larvae do not survive the winter; however, under laboratory conditions, it is possible to breed a nondiapausing strain of gypsy moth (Hoy 1977). For the majority of individuals in a field population, diapause is both obligatory (Leonard 1968b) and genetically determined (Goldschmidt 1934). The induction of diapause is unaffected by photoperiod (Leonard 1968b).

Diapause and Hatching

It has not been possible to separate the diapause and hatching requirements of gypsy moth eggs

because the only published measurements of diapause have been inseparably linked to hatching. Thus, any conclusions about diapause must be based on either percent hatch or incubation time of eggs.

For those gypsy moth eggs that enter diapause, hatching is delayed for at least 60 days. Efforts to terminate diapause by manipulating photoperiod and temperatures do not cause hatching in fewer than 60 days (Leonard 1968b); neither do treatments in hydrochloric acid for up to 24 hours (Leonard 1968b). In general, there seem to be both time and temperature requirements for the completion of diapause. A prolonged period of chilling and a subsequent incubation period are necessary for egg hatch by most individuals of a population. The number hatching and the incubation requirement are dependent upon both the extent and the duration of chilling. Further variation in experimental results has
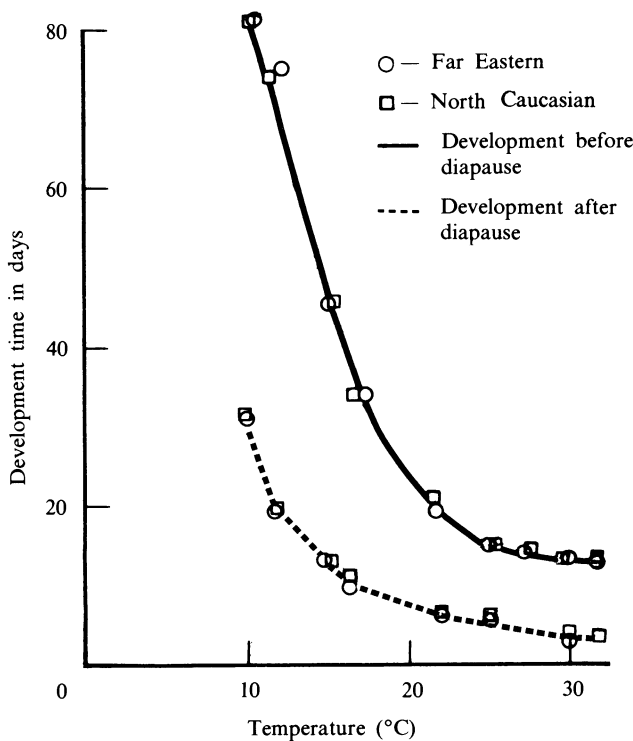
Figure 4-43.—*The effect of temperature on developmental time of gypsy moth eggs prior to diapause (upper curve) and following diapause (lower); far east eggs from Vladivostok (43N 131E) while the north caucasus eggs derived from Kursavka (45N 41E). (Adapted from Pantyukhov 1962.)*

been caused by large differences among individual gypsy moth eggs whose diapause requirements seem to represent a discontinuous distribution from no diapause at all to a diapause of several months.

Survival as a Diapause Indicator

Temperature Effects

The question of temperature effects on mortality during diapause was addressed by Masaki (1956), who held newly embryonated eggs at six temperatures for a period of 80 days and then incubated them at 26° C. The results of these experiments (fig. 4-44) indicate that 5° C may be the optimal temperature for chilling diapausing eggs. Hatching was nearly zero at 0° C and above 20° C, indicating that both lower and upper thresholds exist. Even at 5° C, however, the relatively low survival of 54 percent after 80 days chilling indicates that this is insufficient time for all individuals to complete diapause (although there are always many other possible explanations for mortality). Subsequent work by Maksimović (1958b) indicated 66 percent survival of embryonated eggs exposed to -8° C for 25 days and then incubated at 20° C. Earlier work by Sanderson and Peairs (1913), as reanalyzed by Giese and Cittadino (1977), indicated that no temperature lower than -12° C was favorable for diapause completion. Thus -8° C appears to be near the lower limit for successful chilling at constant temperatures. The upper threshold of 20° C was corroborated by Maksimović (1958a), who found that, although hatching was possible in eggs held from embryonation at 21° C, and even 24° C, survival was low. Maksimović also confirmed that chilling at 5° C for 135 days resulted in the greatest survival.

Time Effects

Not only the temperature but also the duration of exposure determines survival during diapause. Masaki (1956) held newly embryonated eggs at 5° C for exposures of various durations before incubation at 26° C. His results indicated negligible hatching from 0 to 40 days exposure, 50 percent hatch at 70 days, and 80 percent hatch at 80 days exposure. Exposures between 80 and 160 days resulted in a

survival plateau at 90 percent and 130 days exposure. Maksimović (1958b) similarly treated eggs at 5° C for various times and incubated them at 25° C. His results are very similar to Masaki's, indicating an increasing survival between 10 and 150 days chilling exposure. However, he found that survival dropped off in exposures greater than 150 days. Thus, chilling at 5° C for 130 to 150 days seems to result in the best survival of embryonated eggs stored at constant temperatures.

The effects of temperature and duration of cold storage on survival are summarized in figure 4-45, which includes all published results for constant temperature exposures. The contour lines that separate various levels of survival indicate that, in general, survival becomes higher as chilling temperatures approach 5° C and as chilling durations increase. The numbers included on figure 4-45 refer to incubation times required for 50 percent hatch when eggs are emoved from cold storage and incubated.

Incubation Time as a Diapause Indicator

It has been noted that chilling temperatures of approximately 5° C result in the greatest egg survival. However, an incubation period of a higher temperature must follow the low temperature chilling or no hatch will occur. Several authors have found that the incubation time required for hatching decreases as eggs are held for chilling exposures of longer duration. Typical of many results are those of Masaki (1956), who held embryonated eggs at 5° C for various times before incubating them at 26° C. He found the mean hatching time at 26° C to decrease from 21.5 days after 70 days chilling to only 6.5 days after 158 days chilling. This has been cited by Tauber and Tauber (1976) as an example of gradual diapause termination. It has not been determined whether this gradual termination reflects a gradual change within

Figure 4-44.—*Diapause development under different temperature regimes. During treatment, eggs (of Yokohama origin) were held for 80 days and incubated at 26° C. (Adapted from Masaki 1956.)*
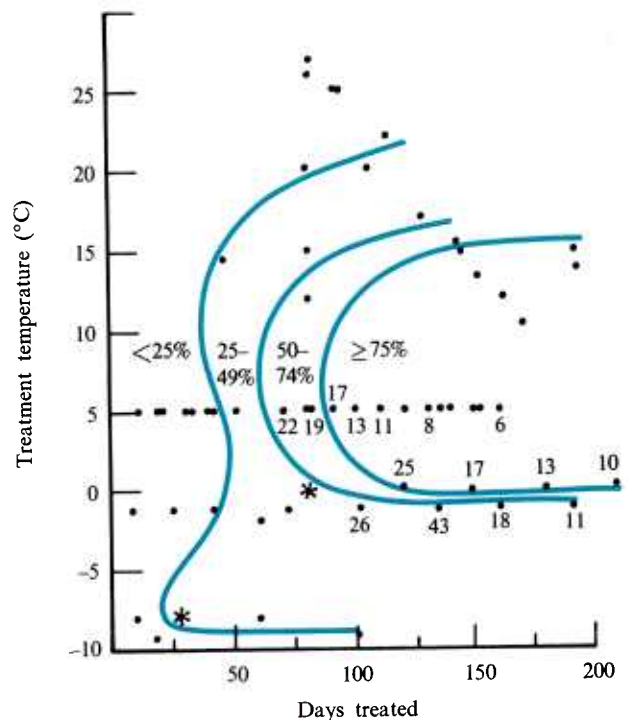
Figure 4-45.—*The effects of constant temperature and time on diapause. Contours separate groups of points with similar percent hatch after incubation (except for the 2 starred items). Values listed at -1° C, 0° C, and 5° C are days preceding 50 percent hatch.*

individuals, or a population change resulting from more and more individuals terminating diapause as chilling continues.

Regardless of the mechanism involved, a declining incubation requirement seems to be associated with the completion of diapause. However, this index of diapause completion is not perfectly correlated with the experimental results that use hatching success as an index. Masaki (1956) showed percentage hatching to plateau at 130 days chilling, but incubation time continued to decline until at least 160 days (fig. 4–46). Zlotin and Treml (1964) showed that under fluctuating temperature conditions a minimum incubation time was reached after 50 days of chilling, and there was some indication that survival was also maximized by this 50-day treatment. Their experiments indicate an accelerated diapause completion associ-

ated with fluctuating temperature conditions. If this is true, diapause may be completed earlier in nature than constant storage conditions indicate.

In addition to indicating diapause completion, the gradually declining incubation requirement is of great importance in the development of predictive models for egg hatch. It seems reasonable to view this gradual diapause termination as a developmental period with a very low threshold. In this light, the results of Masaki (fig. 4–46) indicate that as more development occurs at 5° C, less is required at 26° C. Masaki's results indicate that this diapause development is accelerated with increasing temperature. During the interval between 70 and 90 days exposure at 5° C, the incubation period at 26° C decreases from 21.5 days to 14.7 days. The slope of 0.33 for this interval of the graph indicates that 1 day at 5° C causes the same
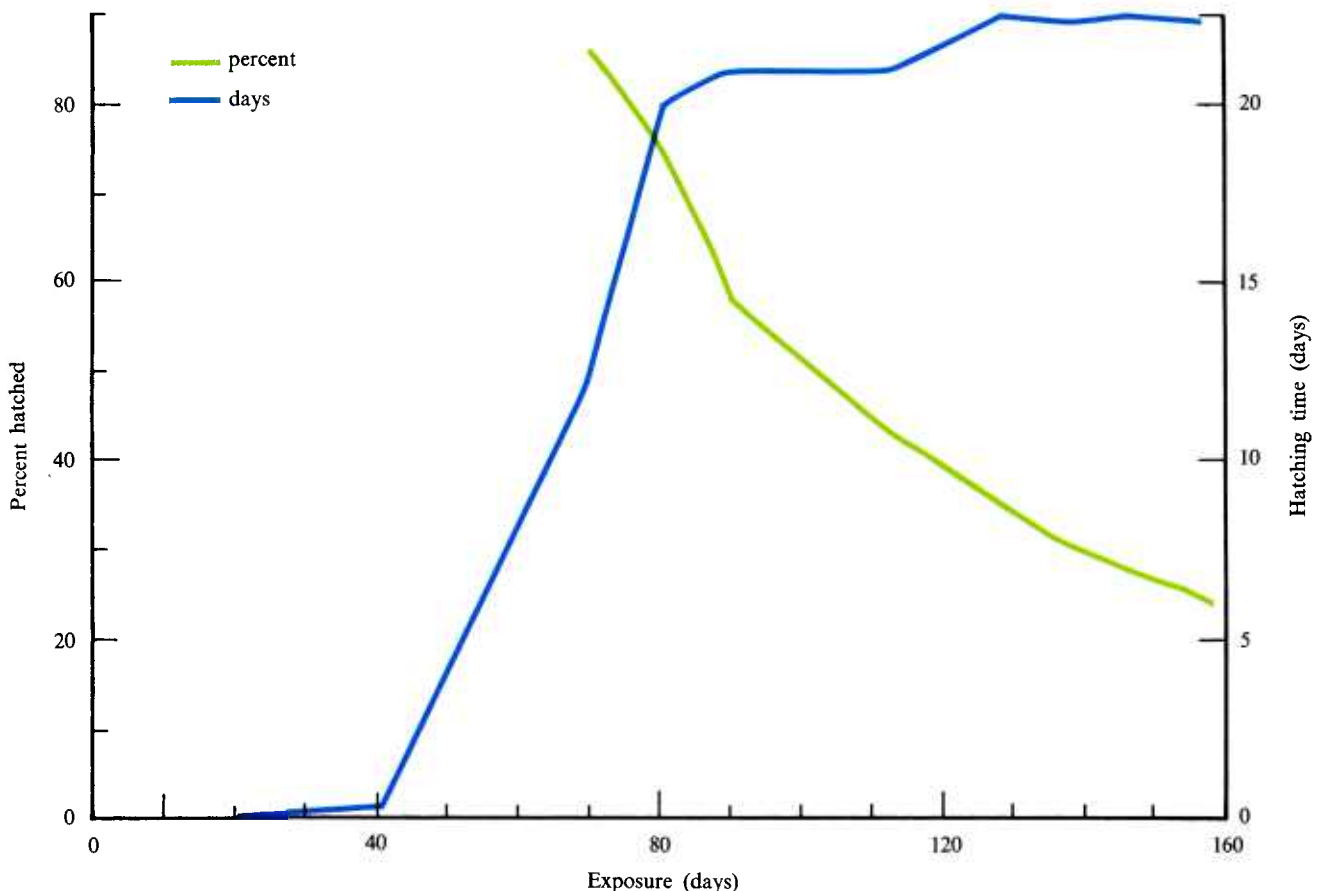
Figure 4–46.—*Hatching success and mean development time of Kitami eggs as influenced by exposure to chilling at 5° C for varying durations; incubation at 26° C. (Adapted from Masaki 1956.)*

progress toward hatching as 0.33 days at 26° C.

This positive temperature response suggests that some measure of accumulated heat units, such as degree days, might be useful in predicting hatching time; however, the curvilinear relationship between incubation time and storage time indicates a much slower diapause development between 128 and 158 days than that which occurs between 70 and 90 days. During the former interval, 1 day at 5° C is only equivalent to 0.088 day at 26° C. Thus, the relationship between 5° C development and 26° C development changes through time. One explanation of this phenomenon is a changing developmental threshold, and indeed, Rubtzov (1938) showed continuous decrease in minimum hatching temperature from about 11° C in February to about 5° C in June. At this point it does not appear that the accumulation of degree days above a single threshold will adequately describe diapause development.

Techniques for Forecasting During the Egg Stage

Defoliation Predictions Based Upon Weather

In the preceding sections, the importance of weather conditions, especially temperature, during the egg stage has been noted. At each developmental phase (embryonation, diapause, and postdiapause) environmental conditons are critical. Not only do they control the rates of development, but they also determine the ultimate success of hatching. At the University of Wisconsin—Madison, this knowledge has been used in constructing generalized models for predicting gypsy moth populations.

Because the data are readily available, defoliation area has been used as an index of population level. Regression models have been established to predict defoliation (Y) as a function of thermal phenomena occurring during the late embryonation period and the diapause phase of the gypsy moth. Specifically, the winter temperature variates used are: Average maximum temperature in November (AMAXTN), average temperature in December (AVTD), and minimum January temperature (MINTJ). In addition, two lagged variables have been incorporated as a "population source": defoliation in years $t-1$ and $t-2$ (DEFt-1, DEFt-2).

The results of applying the above model to data from seven Northeastern States are presented in table 4-38. Only in the Massachusetts model do all weather and lagged variables enter the regression.

The lagged (defoliation) variables are always important, and in a stepwise regression the $t-1$ term is invariably entered as the first step. Note that for Maine, New York, and Vermont, DEFt-1 is the only significant term to enter the equation, accounting for 37–64 percent of the variation in defoliation area at time t. Typically, the R^2 for DEFt-1 is between 35 and 50 percent.

In four of the models, terms representing thermal conditions are present. Higher defoliation is associated with higher average maximum November temperatures. However, average December temperature conditions are inversely associated with subsequent defoliation.

When minimum temperatures in January (often the coldest winter month) are above normal, late-season defoliation is usually greater; other observations and two-thirds of the current data generally confirm this. However, in Rhode Island a negative correlation exists between the minimum January temperature and area defoliated.

During December, most eggs have already entered the diapause phase and higher temperatures appear to be deleterious (considering the signs of the Connecticut and Massachusetts coefficients). Normally, very low January minima are correlated with smaller summer populations, probably due to temperatures near or below the minimum survival threshold. Rhode Island defoliation patterns show a departure from this norm, however, in that higher minimum January temperatures are negatively related to subsequent defoliation levels.

Although area of defoliation is an inexact indicator of statewide gypsy moth population levels, the lengthy defoliation records available provide important clues to population weather relationships.

Egg-Mass Predictions

Other modeling activity at the University of Wisconsin—Madison involves the use of the historic Melrose information (Biging 1978). Of the 40 tree

species present on the Melrose plots, 26 species are present in Wisconsin; these have been assigned to 6 host groups that typify the State's landscape.

Regression models have been formulated for each host group as a function of past gypsy moth population levels and spring and winter variates. Log transformations of the dependent and lagged independent (past egg-mass history) variables are performed to induce homoskedasticity (uniform variance) of the error terms. Although models for the separate host groups provide better precision, results from a common model have been selected for illustration. In this model, egg masses (Y) at time t are predicted by egg masses at $t-1$ and $t-2$, May precipitation ($t-2$), June precipitation ($t-2$), May temperature ($t-1, t-2$), June temperature ($t-1$), and an interaction between May and June temperature ($t-1$).

Two host groups serve to exemplify the model: Oak-hickory (OH), derived from Melrose data, where red, black, and white oak constituted 87 percent of the trees; and the elm-ash-cottonwood (EAC) type that, in this case, was composed of 94 percent elm, river birch, and willow. Considering signs of the coefficients, May precipitation and temperature were positively associated with subsequent egg-mass densities; June variates showed a negative relationship.

Simulations have been run to assess the effect of Wisconsin weather conditions on egg-mass levels. Data were collected for the period 1950–75 for 11 stations distributed nearly uniformly throughout the

State. The simulations were initialized with a medium density of 3,125 egg masses per hectare. The simulations are semistochastic in that the lagged population input levels are modified by the error term; a minimum of 10 replications were performed in this manner.

Under weather conditions prevailing in Wisconsin, simulated populations generally decreased for the first 6 years. At a north-central station (fig. 4–47), egg-mass densities cycled and attained moderate levels twice following the intitial decrease. In the southern part of the State (fig. 4–48), simulated populations showed a similar trend, although the mean egg-mass levels were lower. At both stations the mean density levels of the oak-hickory host group are lower than the bottomland species group. The negative association between June precipitation and egg-mass numbers can be seen in the upper parts of the figures.

When mean egg-mass densities for all years of the simulation at all weather stations are separated into three quartiles, an interesting pattern appears— populations reach higher levels in the northerly locations (fig. 4–49). The simulations indicate that broad geographic areas in Wisconsin can sustain gypsy moth populations.

Low Temperature Effects on the Egg Stage
Ronald L. Giese

The influence of low temperatures on egg viability has been recognized in field observations since early in

Table 4-38.—Relationship of weather during the egg stage to defoliation area for Northeastern States

State	Signs of the coefficients and significance for indicated independent variables										Percent adjusted model R^2 [2]	n years
	AMAXTN		AVTD		MINTJ		DEFt-1		DEFt-2			
	Sign	Sig.[1]	Sign	Sig.	Sign	Sig.	Sign	Sig.	Sign	Sig.		
Connecticut	+	b	–	b			+	b			84	28
Maine							+	b			37	52
Massachusetts	+	b	–	a	+	b	+	b	–	b	78	50
New Hampshire					+	b	+	b	–	b	57	43
New York							+	b			63	23
Rhode Island					–	b	+	b	–	a	67	27
Vermont							+	b			64	22

[1]Indicates significance of individual terms where a is significant at the 95 percent and b is highly significant at the 99 percent levels.
[2]All models are highly significant based on analyses of variance.

this century. Records show that in 1907 whole clusters of gypsy moth eggs failed to hatch in a Massachusetts location (Summers 1922). Friend (1945) attributed the collapse of a 1943 gypsy moth infestation in Connecticut to winter temperatures ranging to –31.1° C. Bess (1961) also noted in 1943 that trees located in Freetown, Mass., showed very little defoliation and that "... the preceding winter had a prolonged period of subzero temperatures which killed about 90% of the overwintering population." Bess further stated that those low freezing conditons occurred on January 20, 1943, and thereafter. On the other hand, Maksimović, Jankovic, and Marcovic (1962) observed that in the winter of 1959–60 an absolute low temperature of –19.8° C in Serbia had no discernible effects on a population of gypsy moth eggs. Low winter temperatures of –18.8° C also caused no egg mortality (Maksimović 1959). According to Kozhanchikov (1950a), gypsy moth eggs can withstand short-term cooling to –45° C for less than a day; however,

longer term cooling periods have a destructive effect. In Leningrad (Kozhanchikov 1950b), winter temperature minima of –50° C had no noticeable negative effects, and Kondakov (1963) observed no mass destruction of eggs in Eastern Siberia associated with minima of –57° C. Benkevich (1963) noted also that short-term temperatures down to –37° C did not influence diapausing eggs. It would appear that somewhere between –18.8° and –31.1° C lie a lethal temperature plus a set of temperatures that, depending upon duration of the exposure, will cause mortality in a fraction of gypsy moth populations.

Simple Temperature Relationships

Clearly, the effects of low temperatures are complex and may be ameliorated by physical environmental, as well as intrinsic, factors; however, the singular action of temperature has been specified in several studies. Among the earliest workers to establish temperature levels related to hatching failure
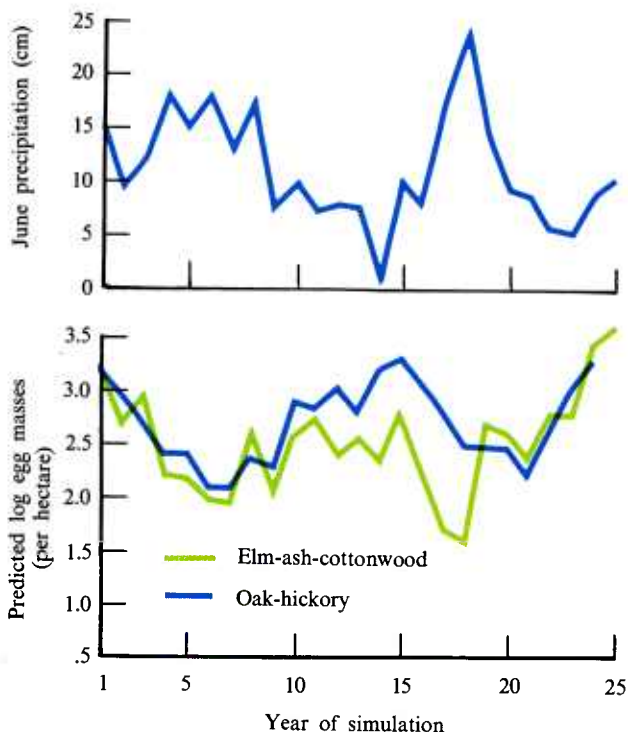
Figure 4-47.—*Mean values of stochastic simulation trials for two host species groups at a northerly weather station (located at A in figure 4-49).*
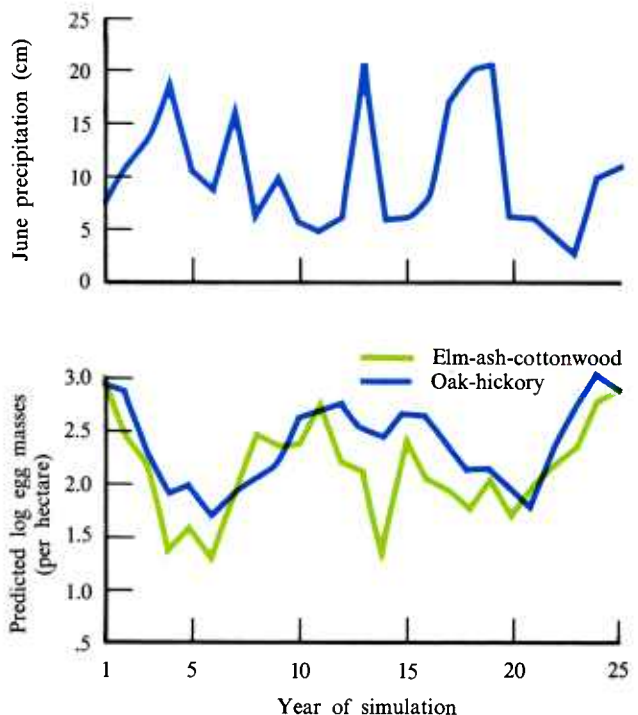
Figure 4-48.—*Mean values of stochastic simulation trials for two host species groups at a southerly weather station (located at B in figure 4-49).*

was Summers (1922), who discovered that a –26.1° C temperature caused 50 percent mortality, and at –31.7° C all gypsy moth eggs were killed. Campbell (1967b) observed that when winter temperatures fall below approximately –28.9° C as many as one-half of the eggs will be nonviable.

Temperatures less than 6° C were claimed by Ali (1933) to be lethal, but his experimental material had been subjected to laboratory conditions and one can assume that diapause had been completed and growth and development had already begun. Hence, in the context of this paper—that is, temperature effects on normal overwintering (diapausing) eggs—Ali's results are inappropriate.

The effects of temperature can be modified by other weather factors; optimal conditions for prediapausing eggs based on temperature and moisture were defined by Kozhanchikov (1950a). The area between the curves in figure 4–50 represents conditions where no mortality could be attributed to atmospheric conditions.

Apparently from information derived by Summers, a –31.7° C level was specified by Brown and Sheals (1944) as the low lethal temperature. Coupled with the knowledge that –26.1° C causes some mortality, these authors concluded that "... regardless of composition, heavy outbreaks will not occur north of a line where at least 25 percent of the years have a minimum temperature of –31.7° C. A contour expressing the distribution of such a temperature-frequency relationship is shown on a map of the Eastern United States in the Brown and Sheals paper. (From a climatological viewpoint, it is interesting to note that the –31.7° C (during one-quarter of the years) contour is similar to a line expressing a mean minimum of –28.9° C.) According to the isotherm presented, northeastern New York, most of Vermont and Maine, and part of New Hampshire would normally be free from gypsy moth outbreaks. Kelus (1941) stated that a January isotherm of –20° C represents the northern boundary of gypsy moth distribution in the USSR. The optimal habitat in
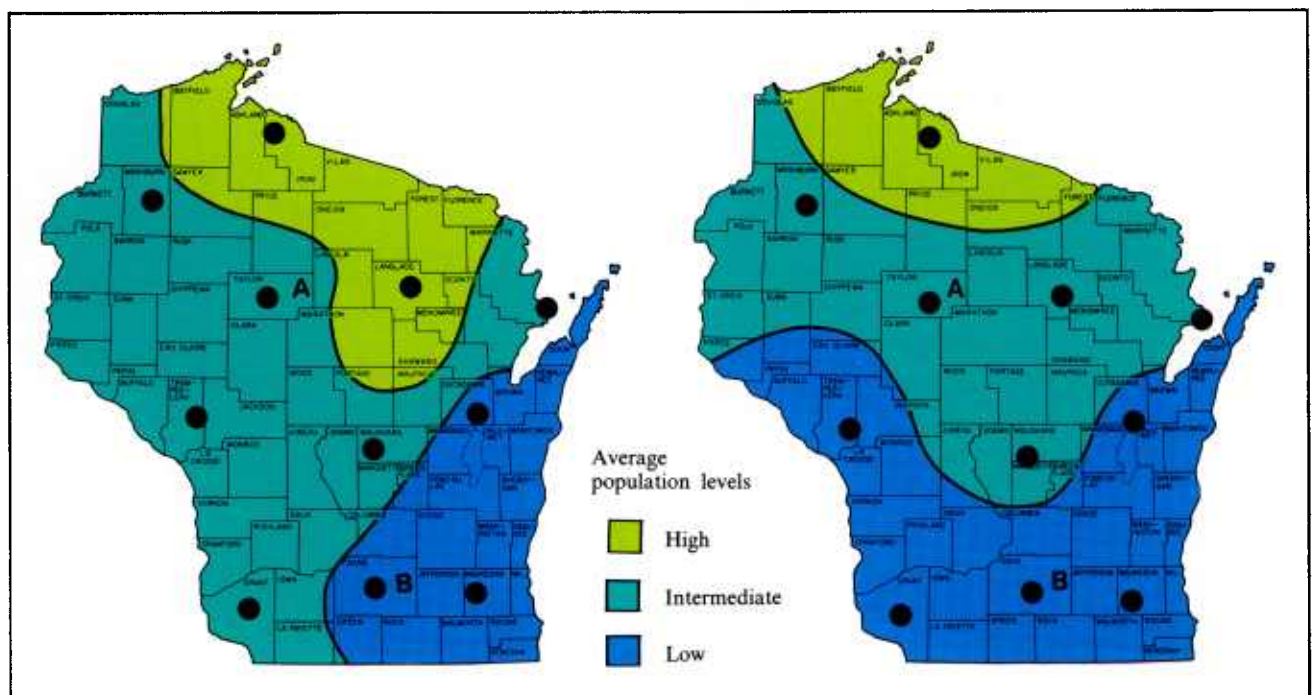
Figure 4–49.—*Susceptibility patterns of two host groups based on simulated gypsy moth populations: Oak-hickory (left map) and elm-ash-cottonwood (right map). Circles indicate location of weather stations used for simulation runs; A and B indicate stations providing data for figures 4–47 and 4–48, respectively.*

Rumania was specified by Simionescu (1973) as an area where the January isotherm is between 2° to 3° C. In a gypsy moth forest system simulation, Valentine and Campbell (1975) used mean minimum temperatures during the coldest month as one of the terms used to estimate subsequent egg-mass density and defoliation.

The best available analytical work on low temperatures was carried out by Sullivan and Wallace (1972) who investigated temperature/mortality relationships of gypsy moth eggs from Quebec and Massachusetts. Based on supercooling point determinations, the initial (control) temperature level causing egg destruction was -28.8° C. However, eggs can increase their resistance to extremes by prolonged exposure to low temperatures (fig. 4–51). (Pantyukhov (1964) recognized also that there was a progression of cold hardiness in early winter and observed that maximum resistance to cold appeared in January to February.) Accordingly, after acclimation, pooled mean supercooling levels became -30.3° and -30.2° C for the Quebec and Massachusetts populations, respectively. The "dosage/mortality" relationships for eggs from the two geographic sources subjected to optimal conditioning temperatures are shown in figure 4–52. Although one-half of the conditioned gypsy moth egg population can survive a short-term temperature treatment of -30° C, extended exposure to that thermal level results in complete mortality after 2 days. This information suggests that at the current level of cold-hardiness, the gypsy moth is likely to expand its range north and westward to include an area bounded by the -30° C mean extreme winter isotherm.

Temperature/Time Relationships

The simple effects of low temperatures on over-wintering eggs can be ameliorated by numerous factors, among the more important of which is the duration of exposure. Working with a European population, Maksimović (1958a) subjected eggs to -25° C for varying lengths of time. Cumulative mortality increased with time at this temperature, and after 5 days 92.4 percent of the eggs were killed (fig.

4–53). Pantyukhov (1964) observed that when eggs from eastern Russia were held at -10.5° C, 50 percent were dead at 110 days, and by 150 days mortality was complete.

Eggs from Quebec and Massachusetts were also subjected to time/temperature investigations (Sullivan and Wallace 1972). The Quebec population responded differently from the control (which was held at 0° C) when subjected to -24° C conditions for 7–16 days. Mortality was 51 percent compared with 24 percent for the control (table 4–39). For the Massachusetts population, a similar significant change from the control appeared when a temperature of -18° C, held for 2–3 weeks, resulted in 41.3 percent egg mortality. With both populations, mortality was 100 percent both after 2 days of exposure to -30° C, and after only several hours exposure at -35° C.

Temperature/Snow Relationships

The insulating qualitites of snow are well known, and gypsy moth populations realize improved survival when egg clusters are covered by snow.

Brown and Sheals (1944) suggested that heavy snowfall, which protects egg clusters deposited on or near the soil, may permit a temporary increase in populations.
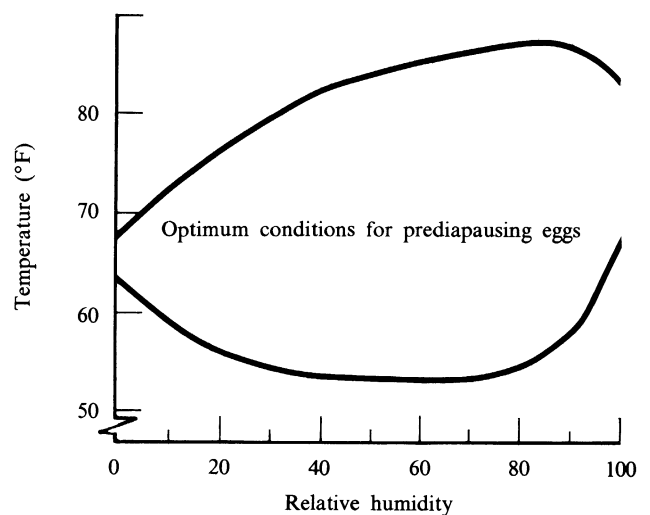
Figure 4–50.—*Thermohydrogram for gypsy moth eggs. (Adapted from Kozhanchikov 1950a.)*

Gypsy moth eggs withstood freezing temperatures to –53° C under snow-covered conditions during 1954–56 in a lowland pine forest in southern Krasnoyar (Kondakov 1961). Eggs protected by snow in Maine during the winter of 1970–71 (Leonard 1972) survived ambient temperatures recorded as low as –32.2° C.

Just how significant snow protection is can be seen in figure 4–54, which shows temperature levels required to kill 50 percent of fully conditioned over-wintering eggs under four snow-depth assumptions. It is obvious that snow depth must be considered as another dimension when examining the distribution potential related to low extreme temperatures.

Temperature/Intrinsic Factors Relationships

Differential responses to temperature can also be caused by the status of a given population. Eggs of the gypsy moth "... cannot survive at temperatures below about –28.9° C," but, under outbreak conditions, egg-mass density is usually reduced to an innocuous level in spring following a winter when the mean minimum temperature was about –17.8° C (Campbell 1973).

Under nonoutbreak conditions, egg density was not particularly responsive to variations in mean minimum winter temperatures higher than –28.9° C, and is "... probably not a biologically significant factor within the innocuous population range" (Campbell 1973).

Larval and Pupal Development
Richard A. Casagrande

Over the years several researchers have studied larval and pupal development under laboratory, and occasionally under field conditions. In this section many of these studies are reviewed to develop an understanding of the factors affecting development. Since this review is part of an effort to develop a general phenological model for use in pest management

Figure 4-51.—*The effect of acclimatizing Quebec eggs on the supercooling level. Point C is the control set subjected to 32° F for 18–25 days, whereas the lower point at 32° F resulted from a treatment at 32° F for 59–74 days; acclimation at lower temperatures was for a 7-day period. (Adapted from Sullivan and Wallace 1972.)*
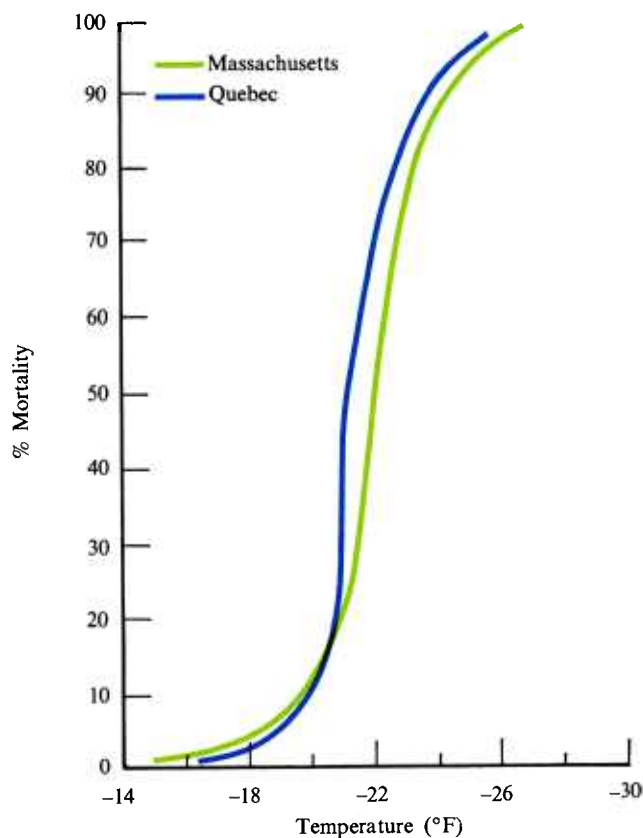
Figure 4-52.—*Cumulative mortality of overwintering gypsy moth eggs from two geographic sources. (Adapted from Sullivan and Wallace 1972.)*

Table 4–39.—*Temperature and time related to mortality of gypsy moth eggs from two geographic sources*

		Eggs from Quebec		Eggs from Massachusetts	
Temperature (°C)	Exposure (days)	Percent mortality	Percent corrected mortality[1]	Percent mortality	Percent corrected mortality[1]
[2]0	18–75	24.0	—	30.3	—
–18	7–11	23.5	0	31.7	2.0
–24	2– 4	24.1	.1	35.5	7.5
–30	2–14	100.0	100.0	100.0	100.0
–35	.3–.8	100.0	100.0	100.0	100.0

[1]Corrected by: $\left(\dfrac{\text{observed mortality} - \text{control mortality}}{100 - \text{control mortality}} \right) 100$

[2]Specified as the control.

Source: Adapted from Sullivan and Wallace 1972.
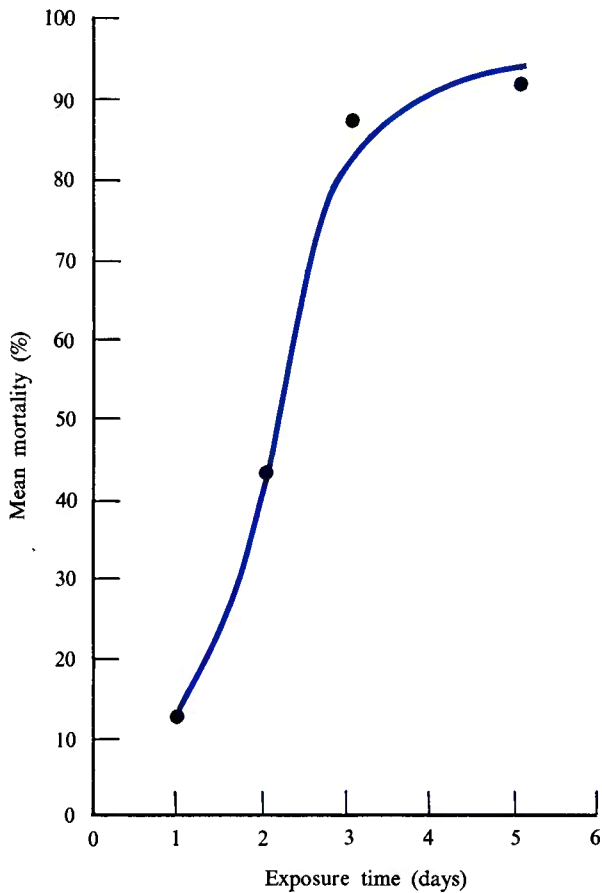
Figure 4–53.—*The effect of exposure time at –13° F on corrected egg mortality. (Adapted from Maksimović 1958a.)*
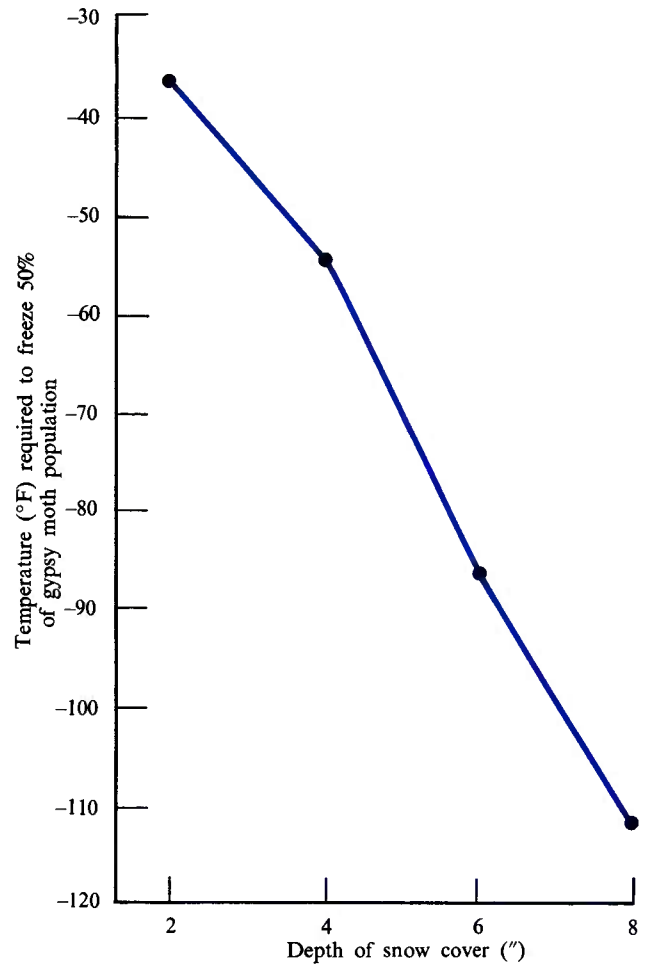
Figure 4–54.—*The insulation influence of snow affecting microhabitat temperatures. (Adapted from Sullivan and Wallace 1972.)*

Table 4–40.—*Summary of temperature-related mortality of gypsy moth eggs, by source*

Source	Temperature (°C)	Percent mortality/or response	Comments
Summers 1922	−26.1	50	—
Summers 1922	−31.7	100	—
Brown & Sheals 1944	−31.7	Heavy outbreaks avoided	If minimum occurs 25 percent of the years
Sullivan & Wallace 1972	−30.3	Mean supercooling point	For conditioned Quebec eggs
Sullivan & Wallace 1972	−30.2	Mean supercooling point	For conditioned Massachusetts eggs
Maksimović 1958a	−25	[1]13	Yugoslavian eggs after treatment for 1 day
Maksimović 1958a	−25	[1]43	Yugoslavian eggs after treatment for 2 days
Maksimović 1958a	−25	[1]88	Yugoslavian eggs after treatment for 3 days
Maksimović 1958a	−25	[1]92	Yugoslavian eggs after treatment for 5 days
Pantyukhov 1964	−10.5	50	Eastern Russian eggs after treatment for 110 days
Pantyukhov 1964	−10.5	100	Eastern Russian eggs after treatment for 150 days
Sullivan & Wallace 1972	−35	100	Quebec and Massachusetts eggs after 7 hours
Sullivan & Wallace 1972	−30	100	Quebec and Massachusetts eggs after 2 days
Sullivan & Wallace 1972	−24	[1]36	Quebec eggs after treatments for 7–16 days
Sullivan & Wallace 1972	−18	[1]16	Massachusetts eggs after treatment for 14–21 days
Sullivan & Wallace 1972	−24	[1]7	Massachusetts eggs after treatment for 2–4 days
Sullivan & Wallace 1972	−24	[1]24	Massachusetts eggs after treatment for 7–16 days
Campbell 1973	ca. −28.9	No survival	—
Campbell 1973	ca. −17.8	Egg population reduced to innocuous level	Under outbreak conditions

[1]Corrected by: $\left(\dfrac{\text{observed mortality} - \text{control mortality}}{100 - \text{control mortality}} \right) 100$

programs, the factors involved are quantified to the extent that the literature allows. There are many problems in synthesizing nearly a century's data collected on three continents. One reason for this is the number of factors involving gypsy moth development. The primary factors investigated to date include: temperature, sex, food, density, geographic origin, photoperiod, and instar number. Most of these factors have been shown by one or more investigators to influence the development rate in both stages.

In addition to the multitude of factors potentially affecting development, a number of complications are involved. For instance, larval instars have frequently been used as a measure of progress toward pupation of gypsy moths. While this is standard practice with many insects, it is very difficult with the gypsy moth since the instar number can range from five to eight and is influenced by the same factors that affect larval development rates. Because no author simultaneously investigated all of the factors

affecting development, and various authors did not standardize important factors, it is frequently difficult to separate a factor such as geographic difference from differences in food, density, photoperiod, etc.

Many of the apparent contradictions in the literature may be due to inadequate documentation. One possible explanation for this discrepancy is that authors may have used a higher intensity of light which imparted sufficient energy to the pupae to accelerate development. The same types of contradictions and uncertainties are associated with almost every aspect of development that has been investigated by two or more authors.

Instar Variability

The larval stage of the gyspy moth normally consists of five instars for males and six instars for females. Each of these instars is described in detail by Forbush and Fernald (1896), who also describe a

seventh instar and note instar variability between and within the sexes. They report that of larvae reared in the laboratory on apple leaves, 26 percent underwent an extra instar and 18 percent had fewer than the "normal" instar number for their sex.

Leonard (1968a, 1970a) reported that rearing temperature affects the incidence of extra instars and that the incidence of extra instars of both sexes was increased by crowding larvae into petri dishes. He found that larvae reared through the first instar at 18.5° C had twice the frequency of extra instars as larvae reared at 23.5° C. In the same paper he noted that there are considerable differences between egg masses in the percentage of larvae to undergo additional instars, and these differences are apparently related to nutrition in the parent generation. Food availability to the larvae also determines instar number (Leonard 1970b). Larvae that were starved during the first instar had a significant increase in extra instars, from about 14 percent for continuously fed larvae to over 30 percent for larvae with 2 days starvation. Thus, instar number is related to larval density, larval food, sex, temperature, and nutrition of the maternal generation.

There is some evidence of geographic differences in instar number of gypsy moth larvae. Goldschmidt (1934) discussed geographic races from around the world with various combinations of fifth- and sixth-instar requirements for both sexes. He also found instar number to be under genetic control. Because of many complications, it is apparent that, for gypsy moths, instar characterization is not a good index of larval development (particularly after the fourth instar). Thus, instar-specific development will not be discussed to any extent in the subsequent sections, but the reader is referred to Maksimović (1958a) for development rates on excised oak leaves.

Temperature Effects on Larvae

As might be expected, temperature is of paramount importance in determining larval development rate. A synthesis of the results of constant temperature experiments by Kozhanchikov (1950a), Maksimović (1958a), and Pantyukhov (1962) shows that larval development rate increases with increasing temperatures between 12° C and 28° C. Above 28° C, increasing temperature did not seem to further increase development rate, and larvae did not complete development at temperatures above 32° C or below 12° C.

Maksimović (1958a) asserted that variable temperatures cause more rapid larval development than equivalent constant temperature exposure. This is at variance with results generally reported on other insects. An examination of his experiments indicates that one of his variable temperature sequences, 21.8° C–31.6° C, exceeds the upper boundary for linearity in the temperature-development rate response. The other sequence, 5.3° C–31.6° C, exceeds both the upper and lower bounds for linearity, and thus neither sequence should be compared to an average temperature as did Maksimović.

Additional research is required to resolve the question of variable temperature effect on larval development.

Sex Differences in Larval Development

All authors who have published development rates for gypsy moth larvae seem to agree that females take longer than males to complete development. This sex difference was quantified by Hough and Pimentel (1978), who showed male larval development to average 15 percent shorter than females, and Kozhanchikov (1950a), who showed a similar 17 percent difference.

Geographical Differences in Larval Development

Three authors have studied gypsy moth larvae from different regions, looking for possible differences in development rates. Goldschmidt (1934) compared larval development rates of populations collected in two areas of Japan and one from Massachusetts. His graphical results comparing total

larval duration indicate a difference of about 15 percent between males from the two sites in Japan and a 30 percent difference between the females from the same sites. The Massachusetts larval duration fell between the two Japanese populations.

Pantyukov (1962) compared several aspects of two populations from the U.S.S.R. His results indicate that a North Caucasus population required about 5.3 percent less time to develop than a Far Eastern population held under similar laboratory conditions. An interesting point is that, based on the climatic data he presents, the warmer climate had the faster developing larvae. Neither the average annual temperature difference (3.9° C) nor the development rate difference is very large, and Pantyukhov stresses the similarities, not the differences, between the populations.

Leonard, in comparing North American populations reared under controlled temperatures on artificial diet, found that three egg masses collected in Quebec, Canada, produced larvae that developed more rapidly than did the larvae from five egg masses from two sites in Connecticut. A reanalysis of Leonard's tabular results shows that fifth-instar males from Canada require 9.3 percent less time than similar Connecticut males for total larval development. A similar comparison of sixth-instar females shows a 7.9 percent faster development of the Canadian population. Leonard speculates that these differences may represent an adaptation to the more northerly climate of Canada. Leonard cited Goldschmidt's 1934 results which supported the concept of more rapid development in colder climates. However, he did not mention Pantyukhov's 1962 work, which showed, if anything, the opposite results in the U.S.S.R.

Thus, as with so many other aspects of gypsy moth biology, the literature is inconclusive on the question of regional differences in larval development.

Effects of Food Type on Larval Development

The most recent published work on how larval diet affects the development rate of gypsy moth larvae is by Hough and Pimentel (1978), who have compared development rates under controlled temperatures on foliage from several host plants. Larvae developed most quickly on red and white oaks and most slowly on red maple and hemlock. (The red oak larvae developed 31 percent faster than the hemlock larvae.) Beech and sugar maple resulted in intermediate development rates. Hough and Pimentel also showed that larvae fed older foliage in June and July developed slower than larvae that initiated feeding in early May (when eggs were naturally hatching in the field). These late-fed larvae took longer on all three hosts tested (white oak, red maple, and hemlock), averaging 21 percent longer development periods.

These results have obvious implications for both laboratory research and field predictions of larval development. In laboratory rearings to determine development rates, it is important that both the type and age of foliage be standardized. In field predictions, stand composition will apparently affect development rates. Thus, an oak stand may experience a more rapid larval progression than a stand of red maples.

Density Effects on Larval Development

Leonard (1968a) observed that in dense populations gypsy moth adults begin to emerge sooner than they do in sparse populations. One possible explanation for this phenomenon is an accelerated development with increased density, and indeed, that is what Leonard found to occur under laboratory conditions. Leonard reared larvae in petri dishes on artificial diet at densities which varied in larval instars from 25 first-instar larvae to three seventh-instar larvae per dish. The development rate of these "crowded" larvae was compared to larvae reared individually in petri dishes. Leonard ran two tests and in each compared the development times of individual versus crowded larvae for fifth-instar males, sixth-instar males, sixth-instar females, and seventh-instar females, a total of eight comparisons. In all cases crowded larvae took less time to develop (five differences were statistically significant).

Prepupal Stage

Toward the end of the last larval instar, gypsy moth larvae stop feeding and enter a quiescent period. They then spin a cocoon formed of a few coarse silk threads where they remain generally motionless until pupation. The time spent in this cocoon before pupation has been referred to as the prepupal stage (pronymphal stage of Maksimović 1958a). Most researchers have included the prepupal stage with the larval stages in measuring development times (as Pantyukhov 1962).

Maksimović (1958a) recorded prepupal development separately and showed it to be temperature dependant, and of short duration (3.15 days at 15.5° C, the coldest temperature giving results in his studies).

It is not known what factors other than temperature affect prepupal development. Maksimović kept data separate by sexes, and his results showed that males generally took longer than females (four out of six temperatures). However, the differences were so slight (less than 1 day) that he did not even mention them. Other factors such as food type, variable versus constant temperatures, etc., may cause differences in duration of the prepupal stage, but these are probably not important because the stage is so short that differences would be measured in terms of hours.

Temperature Effects on Pupae

Several authors have investigated the effects of constant temperatures on the duration of pupal development. The collective results of Maksimović (1958a), Pantyukhov (1962), Kozanchikov (1950a), and Hough and Pimentel (1978) indicate a strong temperature dependance in pupal development between 15° C and 28° C. The developmental response of pupae to temperatures above 28° C is contradictory in the literature. Maksimović's results indicate an upper threshold of 28° C; however, the results of Pantyukhov (1962) indicate a threshold of at least 32° C.

The relative impact of constant versus variable temperatures on pupae has not been well docu-

mented to date. Maksimović (1958a) made two comparisons of constant temperature predictions versus pupal development under natural temperature conditions. He found a slight acceleration in one case and a slight deceleration in the other. Both the absolute maximum and minimum temperatures were well within the upper and lower bounds for development.

Sex Differences in Pupal Development

Of the many factors evaluated for their impact on pupal development, the difference between sexes has been most thoroughly documented. Maksimović (1958a) presented data showing males to complete pupation about 12 percent slower than females, and Hough and Pimentel (1978) showed a similar difference of 15 percent. Thus, it is well documented that males take 12–15 percent longer to complete pupation than females.

Effect of Food Type on Pupal Development

Larval food type does not seem to have a large impact on pupal development rate. Hough and Pimentel (1978) looked at the impact of six host plants on pupal development and found differences which were very small and inconsistent.

Geographical Differences in Pupal Development

Pantyukhov (1962) looked for differences in pupal development between two populations (one from North Caucasus and one from the Far East) and found them to respond almost identically to various temperatures. Thus, on the basis of these two experiments, it would appear that if there are any regional differences in pupal development, they are slight.

Density Effects on Pupal Development

Larval density has been implicated as a factor contributing to pupal development rate. Leonard (1968a) exposed larvae to different densities (as pre-

viously described) and measured the resulting larval and pupal development periods. He found that when larvae were reared under lab conditions on artificial diet, the pupae resulting from crowded cultures developed more rapidly than those from individually reared larvae. Although this difference only averaged 5 percent, it was consistent in all his studies and generally statistically significant.

Future Research

It is possible that additional factors which have not yet been investigated may also affect larval and pupal development and as new results develop, new hypotheses may synthesize the diverse results now available. Research currently underway at the University of Rhode Island is intended to resolve some of the discrepancies in the literature and to quantify the factors affecting development of the gypsy moth. In this research the impact of variable temperatures on larval and pupal development is being carefully investigated because of its importance to the gypsy moth program and to phenological modeling efforts on other insects. Since the developmental response of larvae and pupae is uncertain near the upper and lower thresholds, these temperature ranges are also being examined in growth chamber studies. A phenological model will be developed based on these studies and validated by field studies conducted in Rhode Island and Connecticut. In addition to evaluating the models predictive capability for larvae on white oak foliage, this field study will include several other host trees to further quantify the impact of food type on larval and pupal development.

Summary

It would be convenient if a single value relating gypsy moth egg response to temperature minima could be specified to apply to all populations under all conditions. Except that a low lethal temperature can be given, the complexity and number of interacting factors preclude a simple statement of temperature/mortality relationships. There are, however, important responses of gypsy moth eggs to certain environment-

al and intrinsic factors that affect egg viability and, subsequently, spring larval population densities. A summary table of responses with associated qualifications is presented (table 4–40) to summarize currently available information.

Extensive literature on temperature influences on the egg stage exists, but the lack of attention to conditions preceding wintering (whether natural or experimentally imposed) makes a useful synthesis very difficult. Survival of eggs subjected to extraordinarily low absolute temperatures reported from widespread geographic locations suggests that temperature minima may be a less important determinant of normal mortality than traditionally thought. Given the ovipositional behavior whereby a large fraction of egg masses are situated in locations affording the insulating protection of snow, temperature effects are substantially ameliorated in regions normally covered with snow during the coldest winter months. Indeed, Sullivan and Wallace (1972) concluded that host availability will be a more significant limiting factor than temperature in determining the northernmost range of gypsy moth populations in North America. Their work, the most definitive to date, indicates that the gypsy moth is likely to expand its range to an area bounded by the −30° C isotherm.

Genetic and adaptive variations should be expected for both the northern and southern portions of the North American range. In particular, cold-hardiness should be investigated for populations in the extreme northeast. The western range of the advancing population will probably reflect dynamic values of cold-hardiness that will become important with establishment to the north and south.

Perhaps a more important consideration in egg survival may be maximum winter temperatures, particularly after diapause completion. Periods of warming followed by freezing conditions could have a deleterious effect on eggs. Research efforts should be directed to this phenomenon because of its potential importance in determining establishment and expansion of populations along coastal areas and the southern part of the range.

Larval Dispersal of the GypsyMoth

Conrad J. Mason and Michael L. McManus

Introduction

Dispersal of newly hatched larvae is an important process in the population dynamics of the gypsy moth, although historically most of the emphasis has been placed on its role in the natural spread of the insect. This section provides the reader a brief overview of the importance of dispersal in arthropods and a review of the literature on gypsy moth dispersal. It also describes in detail the approach used to elucidate the role of dispersal in the spread of the gypsy moth, beginning with the development of a conceptual model and resulting in the development and field evaluation of an atmospheric dispersion model.

Dispersal in Arthropods

Dispersal has been defined as the movement away from a populated place that results in the scattering of at least some of the original population (Elton 1947). Although dispersal is constantly occurring in most animal populations and may have minimal effect, mass dispersal may completely change the structure of a stable population or result in the redistribution of a species beyond ecological barriers.

The innate tendency to disperse seems to be present, to some degree, in all species of arthropods and may be accentuated by crowding, hunger, actions of predators, or adverse meterological conditions (Andrewartha and Birch 1954). Most species of arthropods devote a large portion of their collective energies to the founding of new colonies. Small species that are weak fliers or wingless may rely on the wind for dispersal; however, they often possess either large fecundities or pass through many generations per season to offset the low probability of survival characteristic to this method of dispersal. Conversely, larger species may be better equipped with locomotory organs or may possess well-developed sensory organs that enable them to search more efficiently.

Southwood (1962) recognized two types of insect movements—migratory and trivial. Migratory movements take an individual away from its habitat and frequently result in an increase in the mean distance among individuals of the source population. During migratory movements, the individuals will not respond to food, a mate, or habitat, but they will respond to an external stimulus such as light. Migratory flights usually involve the larger winged species that are strong fliers and that can sustain flight for at least an hour.

Trivial movements are usually restricted to an insect's habitat and lead only to a limited increase in the mean distance between individuals. The duration of a trivial movement is completely variable and may be terminated at almost any time on perceiving a vegetative stimulus.

In general, species are categorized as being either actively or passively involved in the dispersal process. However, as knowledge about the dispersal behavior of individual species increases, it is clear that most species are in fact very actively involved. Southwood (1962) uses "passive movement" to refer to those individuals that have no control over the direction or the duration of their flight. This certainly applies to wingless forms such as spiderlings, mites, and small lepidopterous larvae. However, by definition, it would also apply to those winged forms that are carried by convection to altitudes where the temperature is limiting to flight, and that are flying at low altitudes when the surface winds exceed the flight speed of the insect. Therefore, "passive" should refer to the state of the airborne individual and not to the dispersal process common to a species. There are many intricate behavioral mechanisms involved in the release of arthropods into the air, whereupon they are passively dispersed. This is typical of the larvae of the gypsy moth, spruce budworm, and Douglas-fir tussock moth, as well as winged insects such as aphids and leafhoppers.

The literature on the dispersal of insects is voluminous but fragmented. McManus (1979), in a selected review of the literature, discusses the wide range of factors that affects the process of dispersal in

the arthropods. Most of the effort has been devoted to confirming the occurrence of long-distance dispersal of species, but, with some exceptions, little is known about dispersal behavior, the factors that are involved in the release of individuals into the atmosphere, or the factors that either limit or enhance the magnitude of dispersal.

An excellent base of knowledge exists on the dispersal behavior of aphids (Kennedy 1950, Jensen and Wallin 1965, Johnson 1954, Taylor 1965) and on the long-distance dispersal of leafhoppers (Nicki-patrick 1965, Wallin and Loonan 1971). Dispersal is of paramount interest in both these families of insects because they are known to transmit disease organisms (viruses and mycoplasmas) that cause 90 percent of all plant diseases that are vectored by insects (Watson and Plumb 1972). Therefore, the potential impact of dispersal is substantial and contributes to significant economic losses. In a series of papers, Wellington (1945a,b,c,d) discussed the various atmospheric processes important to the vertical and horizontal distribution of insects in general. There are many intricate behavioral mechanisms involved in the airborne dispersal of immature spiders (Duffey 1956, Richter 1970), and some of these factors will be discussed later because they are relevant to observations that have been made on gypsy moth dispersal.

Glick (1939) recovered 30 different species of spiders representing 16 families in a series of flights over Louisiana; one individual was taken at an altitude of 4,600 m. Mitchell (1970) provided an interesting approach to dispersal in mites by comparing adaptations that alter the probability of success in the dispersal of different species. Adaptations may be behavioral or may include developmental traits or reproductive rates that alter the number of dispersers.

Dispersal is an important process in the populations dynamics of all arthropod species. However, with few exceptions, the intricate mechanisms involved are poorly understood, and the role of dispersal in the population dynamics of species has been only grossly estimated.

Review of Literature on Dispersal of the Gypsy Moth

In 1910, experiments were begun at the USDA Bureau of Entomology Gypsy Moth Laboratory located at Melrose Highlands, Mass., to determine to what extent gypsy moth larvae were dispersed by the wind. From these studies, it was hoped that control methods could then be devised that would most effectively prevent spread of the insect. Burgess (1913) initially released newly hatched larvae that had been induced to spin silk in front of an electric fan and noted that a few larvae drifted 6.1 to 9.2 m away. Field experiments were then initiated in many locations in Massachusetts to verify that the young caterpillars could in fact be carried by the wind. Wire-mesh screens treated with tanglefoot were placed in a variety of locations: Salt marshes, tops of water towers, and on rafts in cranberry bogs. In most cases, some larvae were trapped, and the distance the larvae were blown was estimated to be from the nearest known infestation upwind of the prevailing winds for that day. New infestations discovered by egg-mass scouts were usually attributed to larvae carried by prevailing winds that originated from infestations in surrounding towns that were somewhat distant.

With the airborne dispersal of larvae confirmed, the emphasis shifted to determining how far larvae could be blown. Collins (1915) trapped nine larvae on the Isle of Shoals, located 9.6 km off the coast of New Hampshire, and claimed that the larvae actually came from an infestation on Plum Island, Mass., 21.6 km to the southwest. Collins (1917) later erected large screens on the beach at Provincetown, Mass., at the tip of Cape Cod, and successfully trapped larvae that apparently originated from a source infestation located 30 to 48 km across Cape Cod Bay.

In 1932, aircraft flights were initiated to trap larvae at altitudes ranging from 152 to 915 m and to further substantiate the probable occurrence of long-range airborne dispersal (Collins and Baker 1934). Daily flights were made during peak dispersal periods in 1932-33 over heavily infested areas of Massachusetts.

In 1932, three larvae were trapped, one between 91 and 152 m and two at 305 m; in 1933, only one larva was trapped in 14 flight days, but that individual was recorded at an altitude of 610 m. The authors concluded that "the capture of a single specimen at such an altitude leads one to believe that there must have been great numbers floating above the forest crown for at least 610 m above sea level, otherwise the capture of a single specimen should have been extremely improbable."

On the basis of these early investigations and the conclusions reached, windblown dispersal of gypsy moth larvae has always been viewed as a long-range, regularly occurring process. Nichols (1961) reported that prevailing westerly winds and a severe storm in May were probably responsible for distributing larvae 56 km away from an outbreak on Mt. Yeager, Pa. (elevation 610 m).

An overview of the literature on dispersal of the gypsy moth suggests that much of what is known was concluded from inference. The early workers felt that dispersal was enhanced in dense populations of gypsy moth by large numbers of newly hatched larvae searching for food (Burgess 1913). It was also generally accepted that high winds must be accompanied by high temperatures ($\geq 21^\circ$ C) in order to bring about the spread of the larvae. According to Collins and Baker (1934), newly hatched larvae are inactive below 15.5° C and only slightly active between 15.5° to 21° C. It is now realized that larvae are active at temperatures below 10° C (Leonard 1971b, McManus 1973a).

In their zeal to determine the long-distance spread of larvae between locations, Burgess (1913) and Collins (1915, 1917) attempted to trace trap catches of larvae to source populations on the basis of known infestations and prevailing winds on that day. However, Burgess (1913) stated that only roadsides, orchards, and private estates were scouted for egg masses because it was impossible and too expensive to look at large woodland areas. This suggests that the actual distribution of the insect over a large geographical area in Massachusetts was poorly

defined and that trapped larvae could have originated from very local infestations in adjacent woodlands. In reference to the larvae trapped on the Isle of Shoals that were apparently windblown 9.6 to 21.6 km over water from the New Hampshire coast, Collins (1915) reported that an adjacent island less than 1.6 km away was later found to be infested. One must also question the use of prevailing winds to pinpoint the location of the source population from which the larvae were trapped. Most of the wind records came from the Weather Bureau station at Boston and were far removed from the study location. Additionally, daily averages were cited for both velocity and direction of the wind and must be considered inadequate.

It is of interest that early data reported by Minott (1922) concerning the dispersal of newly hatched larvae from a woodland onto a cranberry bog in Plymouth County, Mass., actually suggested that most dispersal is short range. Horizontal traps (5 m²) were placed at varying distances from the western edge of an infested woodland that contained oak, birch, and pine. The traps were checked for larvae daily over a period of 13 days. The following tabulation is reconstructed from data contained in the original article:

Distance from woodland edge (m)	Total number of larvae trapped
30	465
75	373
180	159
275	113

The author stated that "the largest numbers of larvae were taken from the traps nearest the border of the bog, the number gradually diminishing as the distance from the border increased" (Minott 1922). Actually, 76 percent of the larvae trapped were captured within 75 m of the woodland edge.

The issues that have been raised are as much a problem in this era of technological development as they were at the turn of the century. Gypsy moth populations are still not monitored annually within the generally infested area, and although State and Federal agencies do use aerial reconnaissance to

record defoliation that is associated with dense populations, the distribution of sparse or predefoliating gypsy moth populations is not known for any of the generally infested States.

Biologists frequently use the term "prevailing winds" to explain the downwind directional movement of biological organisms (prevailing means most frequently observed). There is no doubt that, on any particular day at any one location, a tendency exists for the winds to originate from a general direction based on the season and the storm tracks associated with that region. However, within any increment of time, the velocity and direction of winds are in fact extremely variable when actually measured on site. Figure 4–55 is a plot of data recorded with a bivane anemometer on Cape Cod, Mass., during a dispersal study in 1974. These data are 10-minute averages (velocity and direction) measured above the forest canopy for a 70-minute period, which is known to be optimal for larval dispersal. This does not necessarily represent an extreme situation, yet the direction of the wind varied over 73° and the velocity

Figure 4–55.—*Midafternoon 10-minute wind speeds and directions. The wind speed is proportional to the length of the arrow, which is pointing in the direction the wind is blowing. The 10-minute averages are numbered consecutively.*

ranged from 1.1 to 2.6 m per second within a period of 60 minutes. This demonstrates the need to record meteorological data on site and coincident with observations on biological events. It also raises doubt as to the validity of using prevailing wind data to establish the source of gypsy moth larvae that are trapped at distant locations, such as on the Isle of Shoals. One must also consider that wind profiles adjacent to large bodies of water are both complex and variable and involve phenomena such as the "sea breeze effect," discussed later in this section.

All the early studies on larval dispersal centered around the physical aspects of dispersal, because the major concern at that time was to restrict the spread of the insect. A costly barrier zone enclosing 27,300 km² was maintained from 1923 to 1939 in order to prevent spread of the gypsy moth across the Hudson River. Despite this effort and the Federal quarantine that was enacted in 1912, the inevitable occurred. In retrospect, establishment of the gypsy moth west of the Hudson probably came about through artificial transfer of life stages and not by windblown larvae.

Studies conducted since the 1960's suggest that behavior and quality of individual larvae are important in understanding the larval dispersal of the gypsy moth. Leonard (1967) reported on laboratory studies that dealt with the silking behavior of the gypsy moth and how it might affect the dispersal of the newly hatched larvae. According to Leonard, newly hatched larvae do not trail silk unless they are starved; if starved, they become increasingly active and irritable, trail silk as they wander about, and are easily induced to spin down from the foliage. He also proposed that a mechanism exists whereby at high population densities crowding increases the chance of agitation among larvae, which in turn prevents them from settling down to feed. Under these circumstances, larvae are induced to spin down on silk and are then easily carried away by wind.

Along these same lines, Leonard (1968) proposed that crowding can also induce larvae to undergo additional instars. Extra-instar larvae have a longer prefeeding stage, which in turn enhances the probability of their being dispersed from an area of high larval density where food would probably be
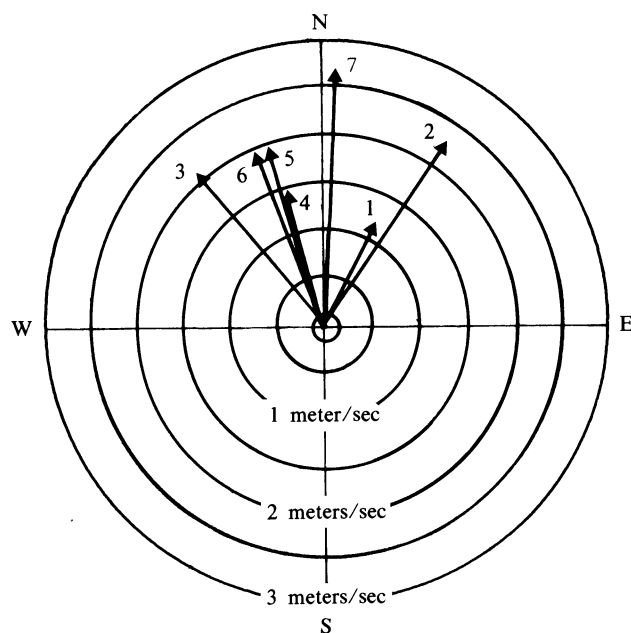
depleted. Leonard (1970a) also suggested that extra-instar larvae could be produced in populations where larvae arise from small eggs that are deficient in yolk. The overall conclusion from the above studies is that a self-regulating mechanism exists in gypsy moth populations whereby a qualitative change occurs prior to population collapse that facilitates dispersal, which in turn gives rise to new outbreaks.

Leonard (1971b) trapped larvae over a 16-day period on large screens constructed in an open field surrounded by woodland that was heavily infested with gypsy moth egg masses. He reported that most dispersal took place in late morning. During the night, at temperatures below 10° C or in periods of rain, larvae were inactive and few were dislodged even by strong winds. He suggested that it might be feasible to manage sparse populations of the gypsy moth to prevent them from reaching a critical size at which density-related effects would induce dispersal.

McManus (1973a) reported on the importance of behavior in the natural process of dispersal of newly hatched larvae. Results of field studies indicated that the larvae always trailed silk when leaving the egg mass and climbing the tree, in contrast to Leonard's earlier results. The rate of ascent and general activity were related to ambient temperature and relative humidity. Hatching, the movement of larvae off the egg mass, and dropping of larvae on silken threads all occurred at specific times in the diel cycle. These activities are similar to those discussed by Edwards (1965) for the Douglas-fir tussock moth (*Orgyia pseudotsugata*). McManus (1973a) concluded that all newly hatched larvae are predisposed to disperse even in the presence of preferred host foliage. Capinera and Barbosa (1976) concurred that all healthy larvae undergo at least an initial dispersal episode. They also found that larger larvae tend to disperse repeatedly; this contradicts Leonard's (1971a) hypothesis that small larvae have a greater vagility because of the longer prefeeding stage and stadium.

The rash of studies on dispersal behavior of gypsy moth larvae has revealed a very complex and fascinating process that involves silking, activity rhythms, and responses to physical stimuli—and the role of population quality on dispersal was still not fully understood. Among the questions that remained to be answered were: How do these findings relate to the actual process of dispersal in the forest? Is dispersal a long-range phenomenon as suggested by the early studies? Would the extent of dispersal be affected by proportions of different-sized larvae in a population as suggested by recent workers?

The stimulus to tackle these questions was provided by involvement in the U.S./IBP Aerobiology Program, one of six coordinated research programs in the IBP. Aerobiology is defined as the study of the atmospheric dispersion of biological materials, although it has been extended to cover related phenomena such as the responses of living material to chemical pollutants and gases. This program was established to bring together an interdisciplinary group of researchers—meteorologists, physicists, biologists, and modelers—at a series of workshops in order to improve communications, assign priorities to research needs in the field of aerobiology, and develop working relationships among the participants. An approach was employed that viewed aerobiological phenomena as ecological systems involving the interactions of biological and physical processes. The objective was to develop simulation models that could be used for predicting or describing dispersion patterns of biological particulates. An excellent example is the EPIMAY model developed by Waggoner and others (1972) to predict the spread of the southern corn leaf blight in the Central States.

Development of a Conceptual Model

The study's objective was to develop a simulation model that would predict the magnitude of gypsy moth larval dispersal from a known egg-mass population. The first step in developing a model was to construct a conceptual model (fig. 4–56) that describes the component elements and their functional relationships (McManus 1973b). A common aerobiological pathway was identified (Edmonds 1972) whereby some source population (field of ragweed, smokestack, fungal asci) releases particles that are dispersed through the atmosphere and then

deposited, causing some kind of impact. The meteorological and biological factors known to affect the various processes are also identified.

Source Population

For the gypsy moth, the number of potential dispersants (larvae) within a source area can be estimated by determining the number of egg masses per unit area and the average number of viable eggs per mass. In reality, the source population can range from a single tree to an area that encompasses thousands of acres of contiguous oak forest. Theoretically, if the vertical distribution of egg masses within the source area could be estimated, the accuracy of the predictions would increase, the premise being that the greater the number of egg masses in the upper parts of the trees, the higher the probability that larvae will disperse from the upper canopy.

Egg Hatch

In the spring, eclosion of eggs is a function of accumulated day degrees over a base threshold temperature, probably near 4°C. It is known from both historical records and recent data that an individual egg mass hatches over a period of 3 to 5 days and that within a local forest area, the egg-mass population normally hatches over a 2- to 3-week period, depending on the degree of exposure of resident masses. Research that is underway to develop a phenological model to predict occurrences such as initiation and duration of egg hatch is discussed later in this section.

Dispersal from the Egg Mass

When hatching begins within an area, the distribution of hatch can be estimated as can the upward movement of larvae from the egg mass. Most larvae may spend up to 24 hours resting on or near the mass before climbing the tree in response to light. The level and periodicity of activity are determined by both biological factors, such as population quality (Capinera and Barbosa 1976), larval age, and rhythmicity, and physical factors, such as temperature, evaporation rate, and solar radiation (McManus 1973a). The newly hatched larvae turn black within a period of 2 to 4 hours. This has a selective advantage in that, as a black body, they absorb solar radiation, which in turn increases their internal body temperature by as much as 6° to 10° C. This allows larvae to be active on early spring mornings when ambient temperatures are below 10° C.

Dispersal from the Tree

The same factors that influence the movement of larvae from egg masses to the tree crown also affect their release from the tree. When larvae are at the periphery of the crown, they continually move around and trail silk but do not feed (fig. 4–57). They respond to light gusts of wind by arching their bodies and then dropping from the foliage or twigs on silken threads (fig. 4–58). If winds are insufficient to fracture the silk threads, larvae will repeatedly climb back up the threads and drop again. Eventually, as horizontal winds and turbulence increase near midday, most larvae that have dropped on threads will have dispersed.
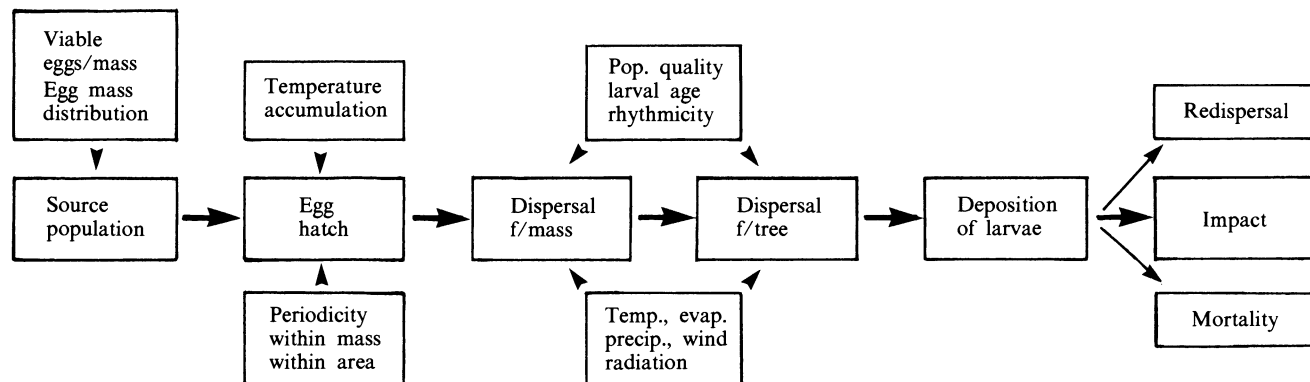
Figure 4–56.—Conceptual model of larval dispersal.

Deposition

Once larvae are airborne, their fate is determined by atmospheric factors such as the turbulent structure of the atmosphere, wind speed, and direction. The relevance of these factors will be discussed in detail later in this section. The passively blown larvae can be considered particulates in the same context as pollen grains, fungal spores, bacteria, algae, or emissions from smokestacks. The only differences are related to the settling velocity of each (a function of their mass), the rate of emission into the atmosphere (entrainment rate), and the extent of the source area.

Eventually larvae will be deposited some distance downwind from the source with the following results: Larvae will land on or crawl onto acceptable host plants and begin feeding; larvae will land on either acceptable or unacceptable plants and redisperse; or larvae will be deposited in lakes or other bodies of water, beaches, pastures, or urban situations from which they cannot redisperse and will eventually die. Mortality at times is probably very high during the dispersal of passively blown species such as the gypsy moth. This particular species is able to compensate for such losses by possessing a relatively high fecundity.

Figure 4–57.—*Newly hatched larvae trailing silk prior to dispersing.*

Miller (1958) estimated the average mortality rate of dispersing spruce budworm larvae in the fall over a 6-year period on the Green River Watershed, New Brunswick, as 71 percent (48–88 percent range), depending on the age, structure, and composition of the surrounding forest. This figure indicates wind dispersal to nonhost material, predation, and failure of larvae to establish. It is reasonable to assume that, in the case of the gypsy moth, dispersal also results in significant larval mortality.

After basic relationships have been established, the next steps in the modeling process are to assign mathematical functions to those processes where data are available, obtain needed data whenever possible, and make reasonable assumptions when necessary.

Development of an Atmospheric Dispersion Model

Atmospheric dispersion models predict the concentrations of materials entrained in the atmosphere that are being dispersed by its turbulent motions. These models are utilized extensively and routinely in air-pollution studies to yield estimates of atmospheric pollutant loadings (Moses and Mason 1975); regulatory actions are often based on their predictions. Input data usually include the locations at which materials are injected into the atmosphere, the entrainment rates, and the prevailing weather conditions, while output data include predictions of concentration and their dependence on space and time. The model itself consists of an algorithm—that is, a set of mathematical equations and procedures for applying them—that best describes the physical processes by which materials entrained in the atmosphere are dispersed. The equations of a model carry with them an implication of a precise knowledge of the phenomenon under study simply because of their seeming exactness. In fact, however, these equations usually are only an expression of imperfect knowledge and represent a drastically simplified world to make the model usable from a practical point of view. Atmospheric dispersion models are not regression models, although these are certainly important to some situations, namely, where either very meager

Figure 4-58.—*Larvae suspended from foliage on silken threads.*

knowledge of the process under study is available or a large number of variables exist that enter in unknown ways. Regression techniques produce purely statistical models evaluated primarily to gain insight into some process that is not simply explainable in terms of basic physical and biological mechanisms. In the case of atmospheric dispersion, enough is known about the physical processes to form a completely deterministic model.

The first four elements of the conceptual model discussed earlier—source population, egg hatch, dispersal from the egg mass, and dispersal from the tree—lead as a unit ultimately to an estimate of the number of larvae that leave the treetop dispersal sites. Considerable experimentation has provided enough information actually to quantify emergence rates, larval activity, and periodicity of dispersal (McManus 1973a). Mathematical functions were assigned to the first four elements and were included in the development of a source function model (Edmonds 1974, Mason 1975) with an output that serves as input to the atmospheric dispersion element of the conceptual model. This results in a completely deterministic simulation model that predicts larval dispersion patterns. Unfortunately, because of manpower and time constraints, verification of the source function model in the field was not possible.

The sole remaining element of the conceptual model—deposition—is concerned in part with the transport of larvae by the atmosphere. Because the larvae have no control over the direction or duration of their flight, their transport is completely "passive." Such passive transport processes are describable by atmospheric dispersion models. Input data are the source functions detailing the spatial and temporal variation of the larval entrainment rate; output data can be maps showing larval deposition isopleths at the end of the dispersion period. In addition, sufficient meteorological information to define wind direction, wind speed, and turbulent intensity throughout the dispersal period must be available as model input. The successful application of such a model to the dispersion of gypsy moth larvae will allow quantitative predictions relative to the range of dispersion.

Gaussian Plume Model

The Gaussian plume model is the best representative example to introduce the concepts associated with dispersion modeling. It is a simple model and perhaps most widely used to estimate pollutant concentrations for level terrain situations. If a ground-level coordinate system is introduced with the x-axis aligned in the direction of the mean wind, the z-axis along the vertical, and the y-axis as the crosswind axis, then the equation that gives the concentration C as a function of distance from an elevated point source can be written:

$$1. \quad C = \frac{Q}{U} \frac{1}{\sqrt{2\pi}\,\sigma_y} \exp\left[-\frac{1}{2}\left(\frac{y}{\sigma_y}\right)^2\right] \frac{1}{\sqrt{2\pi}\,\sigma_z}$$

$$\exp\left[-\frac{1}{2}\left(\frac{z-h}{\sigma_z}\right)^2\right]$$

The quantity Q, the source function, defines the strength of the source (the number of organisms or the amount of material entrained per unit time such as the number of larvae leaving the dispersal sites per minute). Q is assumed constant in time; there is no temporal variation in release or entrainment rates. The factor $1/U$, where U is the mean wind speed, is the dilution factor and expresses the inverse proportionality between concentration and wind speed. The parameters σ_y and σ_z are the dispersion coefficients. They vary with the turbulent structure of the atmosphere, the distance from the source, the sampling time, and the characteristics of the terrain.

The equation is written in this form to emphasize that it is the product of two normal distributions—that is, the bivariate normal distribution. A Gaussian point-source plume from an elevated source is depicted in fig. 4–59. For example, such a plume composed of diffusing larvae can be pictured as emanating from a single large isolated tree, provided one stands far enough from it so that the vertical and horizontal extent of the plume at the origin cannot be seen. (All sources ultimately can be treated as point

sources if the viewer is sufficiently removed from them; additionally, procedures exist to modify equation 1 for area sources and line sources such as large wooded areas or lines of roadside trees.) Either a vertical or horizontal slice through the plume shows a normally distributed concentration pattern in accord with equation 1. Figure 4-60 depicts the ground-level concentration pattern from an elevated source as predicted by this equation. Note that the maximum concentration is not at the base of the source but at some distance downwind, determined by atmospheric turbulence and wind speed.

At any point near the earth's surface, the wind speed and direction are constantly changing. These fluctuations are induced either by direct interference with the smooth flow of the wind by obstacles and surfaces, or by convectively driven circulation cells. This continuous fluctuation, called turbulence, disperses material entrained in the atmosphere. The dispersal process is illustrated in figure 4-61, which shows a puff of material acted upon by horizontal turbulent eddies of various sizes as it moves through the air. In figure 4-61, A, the puff is much larger than the eddies

and is dispersed very little, moving as a unit in the local wind field. In figure 4-61, C, the puff is about the same size as the eddies and both dispersion and a deviation from straight-line motion occur. Figure 4-61 depicts the action only of horizontal eddies on the puff; vertical eddies exist in the atmosphere and act simultaneously to disperse the puff in the vertical direction. Any one puff undergoes all the above dispersive modes simultaneously, because eddies of all different sizes exist in the atmosphere. And, since the puff is changing in size as it is dispersed, the size of the eddies most effective in dispersing it also changes. Eventually the puff is completely dissipated.

The turbulence of the atmosphere can be measured with a highly responsive anemometer bivane; the resulting measurements are related to the magnitude of the dispersion coefficients. The details of this development are provided in Slade (1968), but the dispersion coefficients themselves must be determined experimentally. Field experiments utilize controlled releases of atmospheric tracers impinging on a grid of samplers to measure these coefficients. The magnitude of the coefficients depends on the amount of
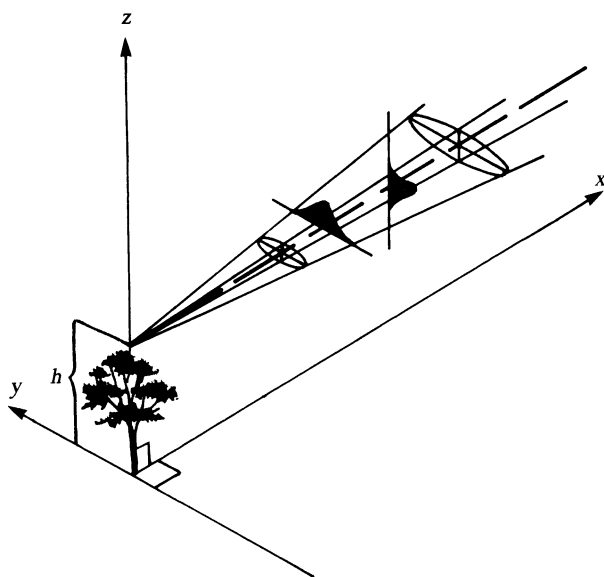
Figure 4-59.—*A Gaussian plume emanating from an elevated source. The shaded curves depict the normal distributions of the concentration vertically and horizontally; the elliptical curves are the loci of equal concentrations. (From Turner 1969.)*

C — Concentration

y — Crosswind distance

x — Downwind distance
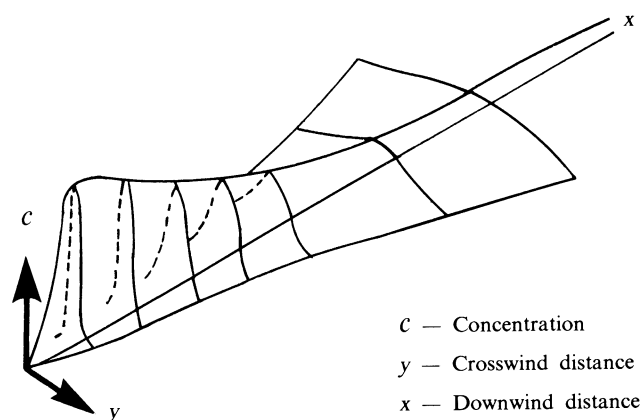
Figure 4-60.—*Ground-level concentration distribution from an elevated source. Concentration is plotted vertically as it depends on downwind and crosswind distances. (From Slade 1968.)*

Table 4-41.—*Relation of turbulence types to weather conditions*

Surface wind speed (m/sec)	Daytime insolation			Nighttime conditions	
	Strong	Moderate	Slight	Thin overcast or $\geq 4/8$ cloudiness[2]	$\leq 3/8$ cloudiness
<2	A	A–B	B		
2	A–B	B	C	E	F
4	B	B–C	C	D	E
6	C	C–D	D	D	D
>6	C	D	D	D	D

A—Extremely unstable conditions
B—Moderately unstable conditions
C—Slightly unstable conditions
D—Neutral conditions[1]
E—Slightly stable conditions
F—Moderately stable conditions
[1]Applicable to heavy overcast, day or night.
[2]The degree of cloudiness is defined as that fraction of the sky above the local apparent horizon which is covered by clouds.

radiant energy reaching the earth's surface (insolation) and the wind speed. If the experimental data are stratified with these two factors as parameters, then universal curves can be constructed that depict the dispersion coefficients as they depend on distance from the source. An example of such a determination is shown in figure 4–62; plots of the horizontal dispersion coefficient σ_y and the vertical dispersion coefficient σ_z are shown as a function of distance from the source, with "atmospheric stability" as a parameter. These graphs can be used in subsequent diffusion experiments or in dispersion model equations like equation 1.

Atmospheric stability is related to wind speed and insolation and can be determined from table 4–41 (from Slade 1968). Strong sunlight and low winds are characteristics of an extremely unstable atmosphere that produces a high degree of turbulence. The dispersion coefficients for this case are always larger than all others at any given distance from the source. At night, or for a more stable atmosphere, the degree of turbulence is less, and the dispersion coefficients are correspondingly smaller. Because the dispersion coefficients are measures of the lateral and vertical dimensions of the plume (and, in fact, are analogous to the standard deviation of a normal distribution), plume concentration will be higher in the more stable

atmosphere because the plume is not spread over as large a volume.

The dispersion coefficients also depend on sampling time—the time period over which the concentration measurements are averaged. The outlines of a plume as a function of sampling time are shown in figure 4–63. The left-hand side of the diagram depicts the plume outlines for the instantaneous plume, the 10-minute-average plume, and the 2-hour-average-plume. The right-hand side of the same diagram depicts the cross-plume concentration patterns for the same time periods. The width of the plume increases as the sampling time increases, while the peak concentration decreases. The dispersion coefficients must also increase with time because they are a measure of plume width. The plots in figure 4–62 are based on a 10-minute average.

Lastly, the character of the terrain—grasslands, a heavily forested area, or an urban environment—affects the rate at which the plume spreads. The plots of figure 4–62 were generated from dispersion experiments on an extensive flat, grassy land. Other studies have been conducted in forested regions (Edmonds 1973), in mountainous terrain (Start et al. 1975), and in urban environments (Turner 1964). Strictly speaking, the values of the dispersion coefficients used in equation 1 should be obtained

from experiments conducted in terrain similar to that for which the estimate is required. For convenience, the graphs of figure 4–62 are used for almost all applications of the model, and model predictions seem not to be adversely affected by this procedure; other sources of error introduce larger uncertainties in the model's output.

Biological organisms and particulates of biological origin are generally removed from the atmosphere by the process of dry deposition. These materials do not remain suspended in the atmosphere indefinitely. With regard to the plume model, it is assumed that the organisms or particles are undergoing horizontal transport and, at the same time, are moving toward the ground at a rate determined by their terminal velocity. Mathematically, settling is incorporated by replacing the variable z in equation 1 with the expression $(z - V_g \times t) = (z - V_g)(x/U)$, where V_g is the terminal velocity, t the transit time to the point x, x the downwind distance, and U the mean wind speed. Pictorially, one can represent this change by drawing a downhill-tilted plume centerline aligned with the particle's trajectory from an elevated source. With this substitution, atmospheric and ground-level concentrations can be predicted for organisms and particulates that settle out at a given velocity if that velocity is known. The concentration at any horizontal surface multiplied by the settling velocity, CV_g, equals the deposition rate of material on that surface—that is, the number or amount of materials removed per unit area per unit time. Obviously, the terminal velocity is an important parameter; its value must be determined for the particular entity whose dispersion is being modeled.

The Gaussian plume model has a number of inherent limitations, as this discussion has just shown: Continuous and constant emission from the source (no variation with time in the entrainment rate), dispersion constrained to a flat, featureless terrain, a fixed atmospheric stability class (no change in the turbulent structure is permitted), and an unchanging mean wind direction and speed. In view of all these assumptions, the Gaussian plume model holds only close to the source when no significant change in parameters occurs. These are severe limitations.

A

B

C

Figure 4–61.—*Turbulent dispersion:* A, *A large puff in a field of small eddies,* B, *a small puff in a field of large eddies;* C, *a puff in a field of eddies whose sizes are approximately equal to its size.* (*From Slade 1968.*)

Advecting Gaussian Puff Model

In addition to the aforementioned limitations of the Gaussian plume model, there are other factors that had to be considered prior to the development of a realistic atmospheric dispersion model. The structure and biomass of a forest induce a diurnal variation in the turbulent intensity above the canopy. Because the vegetation absorbs solar radiation, it becomes warmer than the atmosphere immediately adjacent to it (usually about midafternoon) and warms this layer of air. Since the warmer air is less dense than the air above it, it begins to rise and therefore generates convective turbulence. Any model used to describe

the larval dispersion process must incorporate this variation in atmospheric stability.

The rate at which larvae are entrained in the atmosphere exhibits a diurnal variation. As McManus (1973a) shows, there is a peak in the "silking down" behavior in the midafternoon; this behavior initiates windblown dispersal when turbulent intensity is at a maximum. The coincidence of these two events may not be accidental; dispersing larvae from a given source would be spread over a larger area at this time than at any other time during the day for a given wind speed. Obviously, then, the dispersion model must also incorporate the time-varying entrainment rates of the larvae. Because the model will be required to simulate dispersal throughout the entire hatch-and-dispersal period (usually about 3 to 4 weeks in duration), it must also include changes in wind speed and direction that occur during the daily dispersal periods.

The time-dependence of these factors required the choice of a dispersion model that could allow for such variation. To satisfy these requirements, a mathematical model was developed on the basis of the concept of an advecting Gaussian puff to simulate larval dispersion by the atmosphere. The model treats the emission from a source as a series of instantaneous puffs, each of which undergoes Gaussian diffusion while its center moves with the mean wind. At any point in space, the concentration is determined by adding together the effects of successive puffs.

The advecting Gaussian puff model eliminates many limitations inherent in the simple Gaussian plume model. Entrainment rates or release rates that are time-dependent and that may show diurnal variations can be modeled successfully because the strength of the source can change from puff to puff. In addition, the rate at which a puff is dispersing can change while the puff is in motion; such a change corresponds to a change in the value of the dispersion coefficients that characterize these rates. The dispersion coefficients can change because of a change either in the weather (wind speed and/or insolation) or in the terrain. Finally, since the time history of a puff can be followed for a considerable period, the model is no longer restricted to a localized region; for example, the
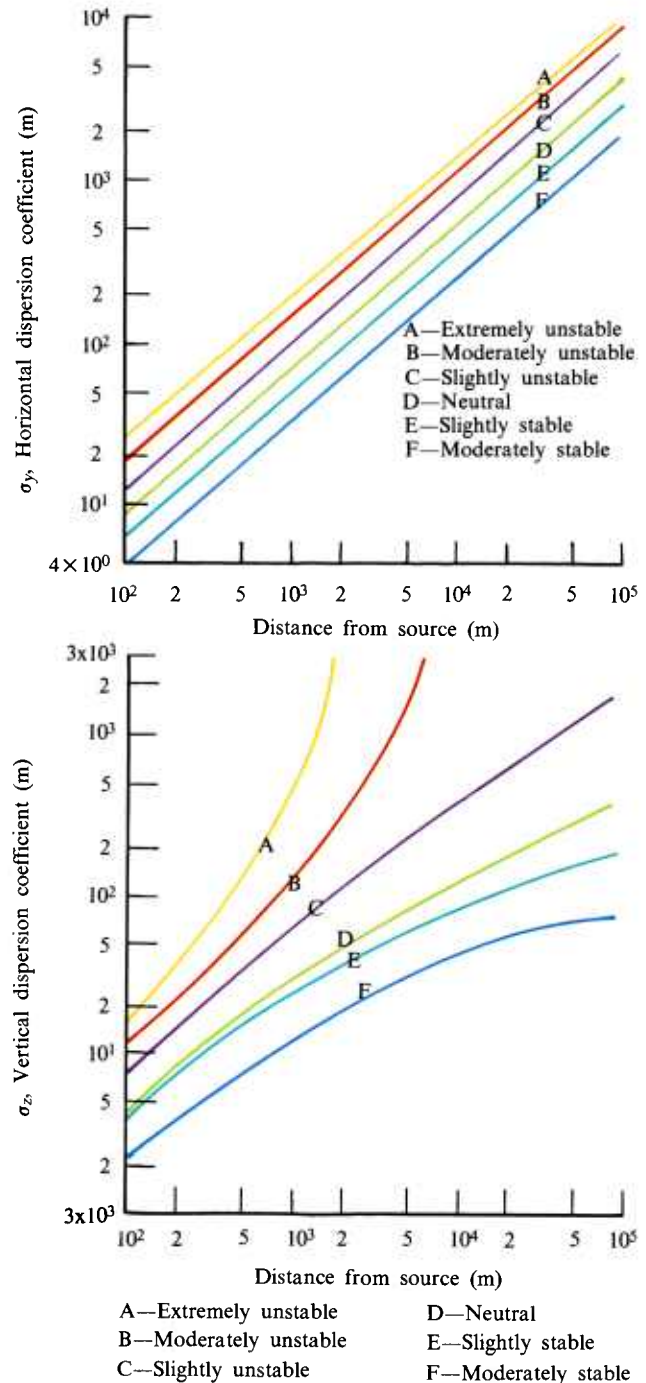
Figure 4–62.—*Dispersion for different categories of atmospheric stability:* A, *Lateral dispersion as measured by the horizontal dispersion coefficient vs. downwind distance from a point source;* B, *vertical dispersion coefficient vs. downwind distance from a point source.* (*From Slade 1968.*)

puff can be considered as moving with mesoscale weather system winds.

The basic equation for the advecting Gaussian puff model describes the dispersion of an instantaneous puff about its center as it moves with the mean wind and can be written (Mason 1975):

2. $C_0(x, y, z, t, t') = Q(t') \dfrac{1}{\sqrt{2\pi}\,\sigma_x} \exp$

$$\left[-\frac{1}{2} \left(\frac{x - U(t - t')}{\sigma_x} \right)^2 \right]$$

$$\frac{1}{\sqrt{2\pi}\,\sigma_y} \exp \left[-\frac{1}{2} \left(\frac{y}{\sigma_y} \right)^2 \right]$$

$$\frac{1}{\sqrt{2\pi}\,\sigma_z} \exp \left[-\frac{1}{2} \left(\frac{z - V_g(t - t')}{\sigma_z} \right)^2 \right]$$

$C_0(x, y, z, t, t')$ is the concentration at time t at the point (x, y, z) owing to an instantaneous release of strength Q at the time t'. The factor $(t - t')$ is simply the time since release or, equivalently, the transit time to the point $(x, 0, 0)$. U is the mean wind speed, V_g is the larval terminal velocity, the x-axis is aligned in the direction of the mean wind, and, as before, y and z are the crosswind and the vertical axes, respectively. No sustained updrafts are assumed to be present—that is, U is completely horizontal.

This last equation is similar to the Gaussian plume equation, especially in the y and z dependence. Dispersion in the x-direction, characterized by σ_x, accounts for the additional factor in equation 2, which is analogous to a normal distribution curve with the mean at $x = U(t - t')$. Because there is no preferred diffusion direction in the horizontal plane, we make $\sigma_x = \sigma_y$. The factor $U(t - t')$ enters as a result of puff movement in the mean wind direction; similarly, the factor $V_g(t - t')$ accounts for the downward motion of the puff because of larval settling.

The larval concentration at any point (x, y, z) and at any time (t), owing to an instantaneous release $Q(t')$ at
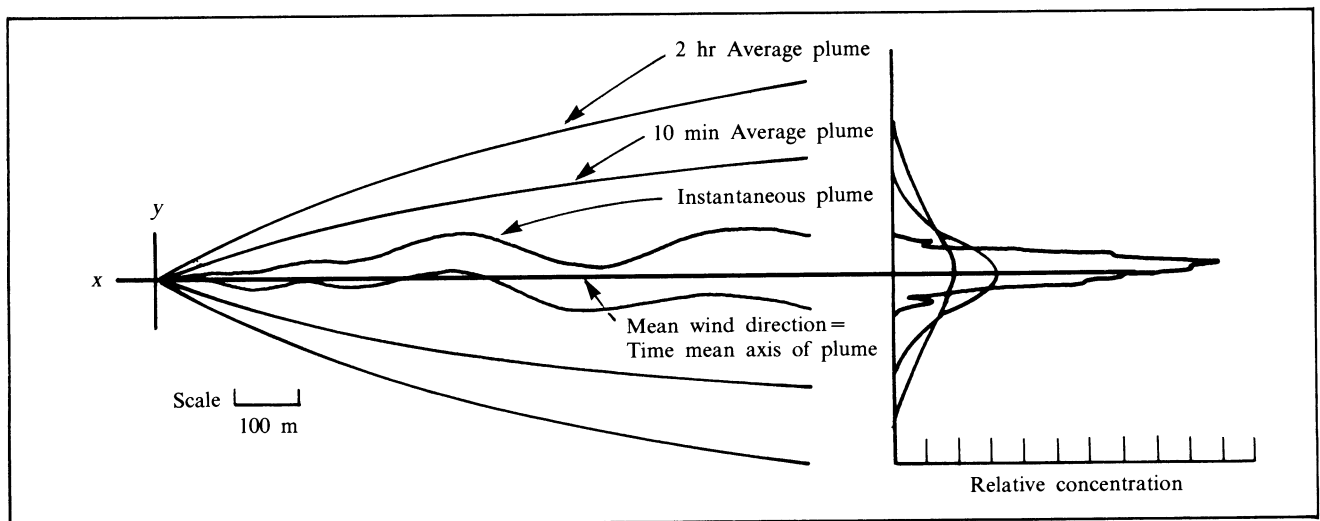
Figure 4-63.—Left, approximate plume outlines averaged over various time intervals; right, corresponding cross-plume average concentration profiles. (From Slade 1968.)

the origin at the time t', is found by adding together the effects of successive puffs. The mathematical statement for this process is:

3. $\quad C(x, y, z, t) = \int_o^t Q(t') \, C_0(x, y, z, t, t') \, dt'$

The integration is performed numerically over 10-minute intervals, assuming that the entrainment rate $Q(t')$ is constant for this time interval and that the wind is steady in direction and speed for the same period. The entrainment rate $Q(t')$ exhibits a diurnal variation and must be known to apply the model for extended time periods. Procedures similar to those of Roberts et al. (1970) are used to extend the model's capabilities to consider varying winds and turbulent intensity as well as to allow application to extended area sources.

As Turner (1969) points out, the Gaussian plume dispersion model provides the best estimates of atmospheric dispersion but not infallible predictions. There are many approximations made in its development, owing to a lack of precise knowledge of atmospheric dispersion under all circumstances. The largest uncertainty resides in the dispersion coefficients; to determine experimentally the dispersion coefficients under all conditions to which the model may be applied is not a simple task and is not usually done. Instead, as noted previously, available values for what may be considered analogous situations are often used. Consequently, a considerable uncertainty characterizes the model output: For dispersion in an unstable atmosphere out to distances of a few kilometers from a given source, model predictions for centerline concentrations are correct to within about a factor of three, all sources of error included.

Conceptually, the advecting Gaussian puff model is a sophisticated version of the Gaussian plume model and can be applied to situations where the latter model cannot be used. However, it shares the same uncertainties with regard to the dispersion coefficients. Thus, in the final analysis, the uncertainty of the puff model is about equal to that of the plume model. Generally, the problem of larval dispersion is constrained to short distances (a few kilometers or

less), owing to the sizable terminal velocities of the larvae. Also, the daytime atmosphere over the forest is usually unstable when conditions are favorable for dispersal. Therefore, predictions should be good to within a factor of three or so.

Determination of the Terminal Velocity of Larvae

As mentioned earlier, certain functions can be approximated; however, the determination of the terminal velocity (V_g) of the newly hatched gypsy moth larvae was immediately identified as a critical requirement in the model (equation 2).

Airborne materials are classified as either gases or particles. Most biological particulates range from 10 to 100 μm in diameter and are usually confined to the turbulent boundary layer of the atmosphere (lower 2 km) (Harrington 1972). Gravity acts upon particles to provide a net settling force. Because of the high surface area per unit mass of small particles and the resulting high air resistance, the particles after a short time will reach a constant velocity at which the force of gravity is exactly balanced by the drag forces. This velocity is called the *terminal velocity* of the particle (sometimes referred to as settling velocity or gravitational fall speed). The drag forces will vary with the character of the flow of air around the particle; therefore the size, shape, and density of the particle will affect its terminal velocity.

Table 4-42 lists some approximate sizes and terminal velocities of biological particulates that are of interest to aerobiologists. Most empirical data on terminal velocities were obtained using regularly shaped glass spheres (Slade 1968). For irregularly shaped particles such as pollen grains, fungal spores, or insects, the terminal velocities are less because of the increased drag resistance of the air. Conidiospores of the fungal genus *Alternaria* have a terminal velocity of 0.3 cm per sec^{-1}, while those of another disease-causing organism, *Helminthosporium maydis*, are reported to have a terminal velocity that is four times that figure (Bell and Burleigh 1973). The gypsy moth larva is shaped more like a cylinder and also possesses patterns of setae (fig. 4-64) that increase the drag resistance. The early workers (Burgess 1913, Collins

1915) discussed in detail the possible role of vesicular acuminate hairs that are found on the newly hatched larvae and even calculated a "soaring coefficient" of the larvae analagous to windblown seeds of plants.

Another factor that must be considered in determining the terminal velocity of gypsy moth larvae is the silk thread that is usually attached to the airborne larvae. This was not considered by the early workers to be as significant as the lateral setae, which are, of course, more noticeable. Recent field and laboratory observations indicate that larvae always released themselves from the foliage with attached silk that then fractures at some point away from its source (the labial silk gland of the insect). As wind velocity increases, the length of attached silk decreases because the silk fractures sooner.

A series of laboratory experiments was conducted in 1974 to determine the terminal velocity of gypsy moth larvae. First-instar larvae varying in weight from 0.5 to 0.8 mg and trailing silk lengths of 0, 30, 60, and 90 cm were dropped from a height of 3 m and the time required for the larvae to reach the ground was recorded. Terminal velocities computed for each category varied from 43 to 132 cm per second, depending on the weight of the individual and length of the attached silk (fig. 4-65). Two important observations suggest that the contribution of silk to the total drag is indeed great: Larvae attached to 90 cm of silk have a terminal velocity one-half that of larvae with no silk, and the orientation of falling larvae will change significantly depending on the length of silk attached. When the silk exceeds 40 cm, the axis of the body remains stationary and parallel to the direction of gravity. This suggests that silk lengths in excess of 50 cm produce a greater drag effect than is produced by the larva complete with hairs.

With a determination of the terminal velocity, a simple trajectory analysis can be used to estimate the projected distance that larvae can be blown from an elevated point source (fig. 4-66). Let h equal the height of the tree canopy (10 m), as in the oak-pine forests of Cape Cod, Mass., and southern New Jersey, and L the distance that larvae will be blown from the top of the canopy if there is no obstruction to lateral movement and air turbulence is minimal. From

Table 4-42.—*Range of diameters and terminal velocities for some biological particulates*

	Diameter (μm)	Terminal velocities (cm/sec^{-1})
Viruses	0.02–0.4	$\leqslant 0.003$
Bacteria	.3–11	.001–.3
Fungus spores	2–100	.003–30
Pollen	10–100	.3–50
Insects and other microfauna	$\geqslant 100$	$\geqslant 30$

regression equations, the maximum terminal velocity of newly hatched larvae (1.5 mg larva with no attached silk) is 132 cm per second; the minimum terminal velocity (0.5 mg larva attached to 90 cm of silk) is 43 cm per second. The time to reach the ground from any height is $t = h/v_g$, where v_g is the settling velocity. Therefore

$$t \text{ max} = 10.0 \text{ m}/0.43 \text{ m/second} = 23.3 \text{ seconds and}$$
$$t \text{ min} = 10.0 \text{ m}/1.32 \text{ m/second} = 7.6 \text{ seconds}$$

The estimated distance traveled in this time interval is $L = (V)(t)$, where V is wind speed in meters per second. At a wind speed of 5 m per second (≈ 10 mph):

$$L \text{ min} = 5 \text{ m/second} \times 7.6 \text{ seconds} = 38 \text{ m}$$
$$L \text{ max} = 5 \text{ m/second} \times 23.3 \text{ seconds} = 116 \text{ m}$$

The most dispersible larva—that is, the smallest (0.5mg) attached to the longest silk thread (90 cm)—will only travel 116 m if blown from the top of a 10-m tree when the wind speed is 5 m per second. Doubling either the wind speed or the height of the tree will result in simply doubling the distance that the larvae will be blown.

If air turbulence is significant and some larvae are lifted to higher altitudes, it will take them longer to settle out in the direction of the mean wind, and the distance traveled will be correspondingly increased. The hypothetical situation just discussed might apply to those larvae that disperse from an isolated tree in the middle of a field or at a woodland edge; in a forested situation, however, there are other considerations. Most larvae that climb trees, drop on silken threads, and are then dispersed probably never reach

the airstreams that flow over the top of a forest canopy. Anyone who has walked through a heavily infested forest at the time dispersal is occurring has observed the gossamer of silk hanging from the branches of surrounding trees. This is evidence that most airborne larvae probably travel no farther than adjacent trees before they get hung up by their silken threads. They may repeatedly redisperse when this occurs, but the total distance covered by an individual is probably not substantial. Only those larvae that are lifted above the canopy or that are blown from the forest edge have a chance to be lifted by updrafts or eddies to heights where they can be dispersed for hundreds of meters or perhaps even kilometers. Therefore, only a small proportion of the total larval population in an area has the potential for long-range dispersal.

Figure 4-64.—*A newly hatched larva showing patterns of setae.*

Model Predictions for an Isolated Point Source

The atmospheric dispersion model described in the preceding section can be used to predict the larval deposition patterns in the vicinity of a given source. Or alternately, its output can be interpreted as the probability of a single larva reaching a specified distance from its dispersal site.

To investigate the dispersion pattern for a simple case, consider a single, elevated source about 15 m high. The wind is blowing at a speed of 2 m per second and is unvarying in direction for a dispersal period of 1 hour, during which time the rate at which the larvae enter the atmosphere is constant at 1,000 larvae per minute. Such a situation might apply to a single infested tree in the middle of a large clearing. The dispersal period is short, so that the effects of larval reentrainment after impact may be neglected.

Figure 4-67 is a plot of the number of larvae deposited per square meter on the ground versus downwind distance from the source. Three levels of turbulent intensity—extremely unstable, slightly unstable, and slightly stable—are compared. A larval settling velocity of 75 cm per second is assumed.

The effect of the large settling velocity is clearly apparent: A threefold decrease in larval area density occurs in a lateral distance of only about 300 m. The effect of atmospheric stability on the dispersion process is not important up to a distance of about 200 m; only for longer range dispersal does stability significantly influence the results. Because an elevated source is considered, the greatest number of larvae are deposited a short distance downwind from the source (compare to fig. 4-60), in this instance 15 m. Obviously, considerable deposition takes place in the vicinity of the source.

Increasing turbulence serves to increase the number of larvae trapped beyond the peak, while at the same time the amplitude of this peak is reduced (fig. 4-67); as expected, larvae are picked up by the more intense turbulent eddies and spread out farther. However, even in this case the reduction in the number of larvae trapped with distance is still large (about 1/1,000 at 800 m compared to the peak value). The crosswind deposition pattern also shows an increase in its width

as the turbulent eddies become more energetic. Figure 4-68 depicts the crosswind deposition 160 m downwind from the source. As before, three stability categories are considered. The slightly stable atmosphere produces a very narrow deposition pattern; the ground-level count decreases by six orders of magnitude in less than 200 m (crosswind) at the selected station. In contrast, the extremely unstable deposition pattern shows a rate of decrease of only about one decade for the same crosswind distance.

The effects of terminal velocity on dispersal range can be investigated by obtaining model output for reduced values of this parameter. For purposes of comparison, deposition patterns for terminal velocities equal to the measured larval rate (75 and 7.5 cm per second, respectively) are shown in figure 4-69. A terminal velocity of 7.5 cm per second is typical for pollen grains; the curves in figure 4-69 are labeled correspondingly. In this simulation, all other values are as before.

As the terminal velocity is reduced, the number deposited at a distance of 200 m or less from the source is diminished. At the same time, the downwind distance at which peak deposition occurs is further removed from the source. For pollen at large distances from the source (about 1,600 m), an increase

in the number deposited is predicted; compared to the larval curve, approximately 10 times as many pollen grains would be found at this distance as larvae.

The crosswind deposition patterns for the two cases under consideration at 160 m downwind from the source are given in figure 4-70. The greater crosswind extent of the pollen pattern (except very close to the plume centerline) as compared to the larval pattern for any given ground-level count is apparent. All these results are a manifestation of the relative ease with which the pollen grains are dispersed by a given level of turbulence because of their smaller terminal velocity.

Although the measured value of 75 cm per second for the larval terminal velocity is large compared to terminal velocities of other materials of biological origin, it is still small enough so that larval dispersion is affected by the level of atmospheric turbulence. Were the terminal velocity somewhat larger, the effect of turbulence would be even less pronounced, and for all practical applications larval trajectories could be computed instead. On the other hand, if the terminal velocity of the larvae were less, deposition near the source would be significantly reduced but correspondingly enhanced at greater distances from the source, in both the downwind and crosswind directions.

Figure 4-65.—Relationship between the terminal velocity (cm/sec) and weight (mg) of first-instar larvae attached to 90 cm of silk (R=98.4 percent).
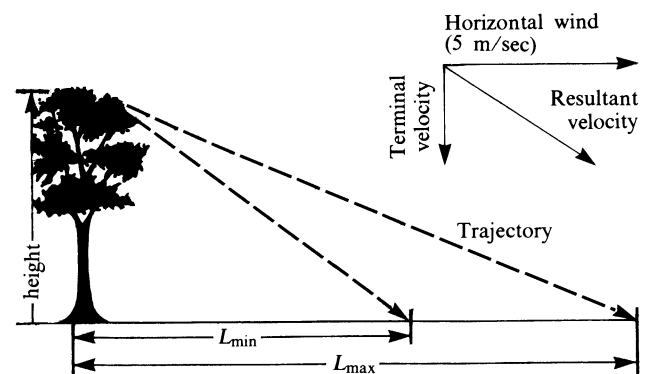
Figure 4-66.—Schematic representation of the factors determining larval trajectories.

The above predictions suggest that for a single larval "hop" the distance traversed is very small, regardless of the turbulent intensity. Because the larvae may actively reentrain themselves in the airstream after impacting and coming to rest for a short period of time on nearby objects, the significant result is that dispersion proceeds by a series of such short hops. It is important to note that these hops are not necessarily all in the same direction—at each successive dispersal incident for a given larva, the wind need not be blowing in precisely the same direction. Although wind directions at successive time intervals are not correlated, they are generally treated as such in meteorological statistics; in that sense, larval dispersion then takes on the nature of the classical random-walk problem in statistics.

As discussed earlier, in the past emphasis was placed on possible long-range dispersion because of instances in which a few larvae have been trapped at great distances out at sea or at great heights in the atmosphere. This model does not preclude such an occurrence, but it does show that the probability of such long-range dispersal is extremely small.

Model Predictions for an Extended Source Region

Dispersion from an extended region can be treated as a conglomeration of point sources distributed over space. However, if the entrainment rate is uniform over the entire region, simplifying assumptions can be made to reduce the complexity of the calculations; namely, a point source located upwind has a plume width at the source center equal to the crosswind width of the source. The computation is reduced to finding the position of this point source (as a function of atmospheric stability) and calculating the deposition from it. An example of the model predictions for an extended region is shown in figure 4–71. The source region is a 10-km square uniformly infested by larvae. Initially, a population of 60 larvae per square meter is available for dispersal. If all these larvae disperse at the rate of one per square meter per minute in a period of 1 hour during which a 2-m-per-second wind is blowing steadily, then the number deposited at the crown level directly beneath the

plume centerline is given in the figure. A moderately unstable atmosphere is assumed (Class B stability), and redispersal is not considered.

Several features of the dispersion from an extended region are apparent. First, at a distance of 4 km from the downwind edge of the source (9 km from its center), the crown-level deposition is one-tenth that of the initial dispersing population (six larvae per square meter). Thus, although the basic dispersal process for a single larva is still short range, the cumulative effect from a large area is significant deposition at some distance from it. Nevertheless, the decrease in deposition is still very rapid; in the next 5 km, the deposition is reduced by another decade to only one-hundredth of the initial density. Another aspect of the
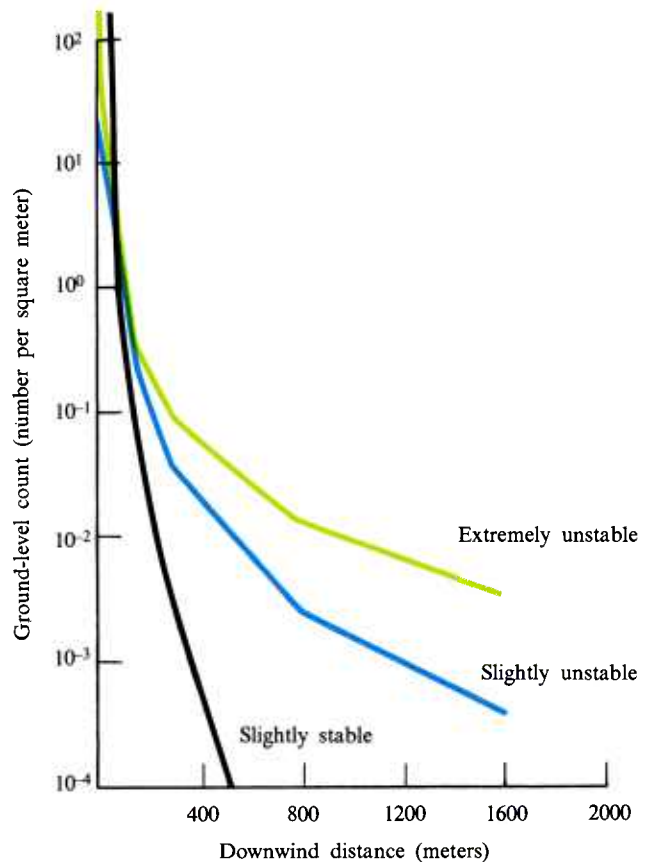
Figure 4–67.—*Downwind ground-level deposition from an elevated point source for a larval terminal velocity of 75 cm per second and for three atmospheric stability categories—extremely unstable, slightly unstable, and slightly stable.*

dispersal process is illustrated quite clearly: The considerable deposition that takes place in the *upwind* direction. Although the same feature applies to a point source, it is more readily apparent for the area source. In this example, a deposition level of one-twentieth the initial level occurs 4 km from the upwind boundary of the infested region. The upwind dispersion is a manifestation of the energetic turbulent eddies that exist in a moderately unstable atmosphere. For the light wind considered in the example, many of

these can be of sufficient magnitude to cause frequent reversals in the wind direction producing the upwind dispersion. These eddies are completely random in nature, and their effects are incorporated in the model through the dispersion coefficients. For a more stable atmosphere or a higher mean wind speed, the curve in figure 4-71 would be skewed toward the downwind direction and less deposition in the upwind direction would occur.

Field Studies to Verify the Model

The Site

Predictions from the mathematical model suggested that in the absence of severe turbulence most larvae should be deposited within 1.6 km of their origin. With this information the model was tested in a natural forest situation in spring 1974. A location on Cape Cod, Mass., near the town of Harwich, was chosen for the following reasons: Topographically, the terrain was level so that the wind profile within the study area could be defined with a single meteorological tower; the land mass around the study area is restricted by water on two sides and thus the chances of infestation from adjacent gypsy moth populations were reduced; canopy height of the forest was only 9 to 12 m, which facilitated placement of the insect samplers at or above the canopy and greatly reduced the cost and effort to install and maintain them; and prior knowledge of the history of the gypsy moth populations in the surrounding area was available because permanent study plots were located in the area.

The study site was a mixed oak-pine forest with a low overstory (10–15 m). Species composition included white oak (*Quercus alba*), red oak (*Q. ruba*), black oak (*Q. velutina*), and pitch pine (*Pinus rigida*). The pines were the largest trees in the stand and overshadowed the smaller oaks, which were more numerous.

Meteorological Instrumentation

A 20 m meteorological tower was installed in close proximity to the study area and was equipped with
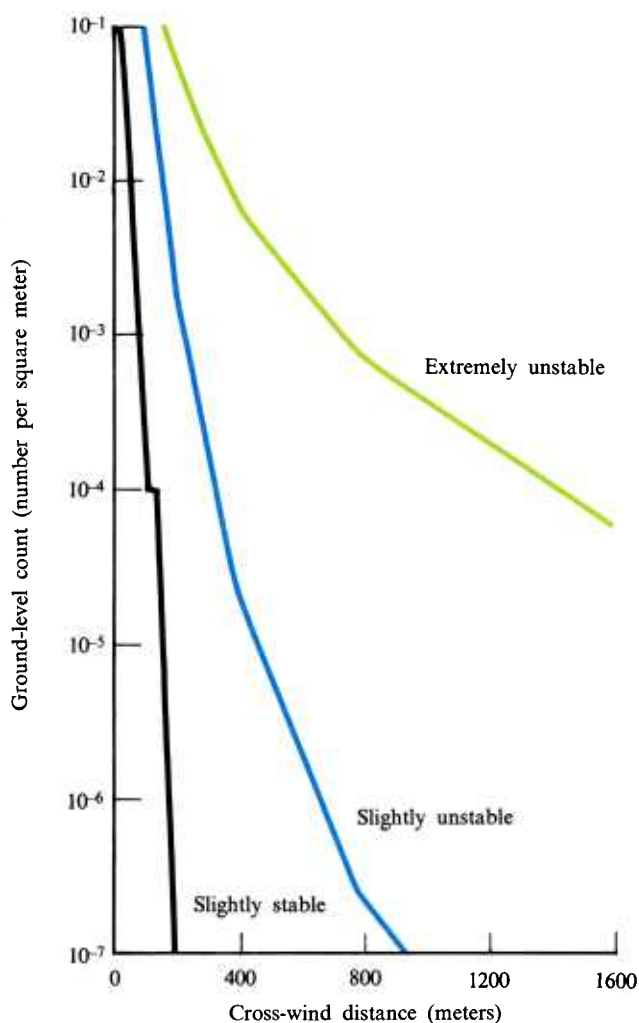
Figure 4-68.—*Corresponding crosswind ground-level deposition 160 m downwind from an elevated point source for a larval terminal velocity of 75 cm per second and for three atmospheric stability categories—extremely unstable, slightly unstable, and slightly stable.*

instrumentation necessary to measure the meteorological parameters required for the model. The tower (fig. 4–72) has booms at 20, 15 (canopy level), and 10 m. Sensitive anemometer bivanes (fig. 4–73) that measure wind speed, direction (azimuth), and elevation (updrafts or downdrafts) were installed at the top and bottom levels. These measurements are directly related to atmospheric stability, which in turn determines how rapidly a plume is dispersed.

Anemometer bivanes are very responsive instruments but are not very durable. As a backup system, temperature sensors in naturally ventilated radiation shields were installed at all tower levels to measure the change in temperature with height (the lapse rate), a procedure that provides another measure of atmospheric stability.
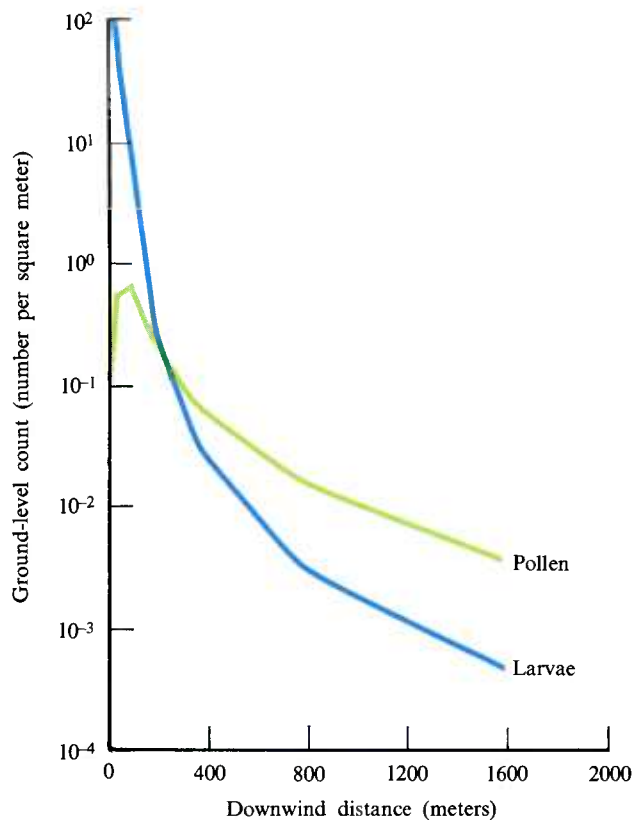
Figure 4–69.— *Downwind ground-level deposition from an elevated point source for a slightly unstable atmosphere and two terminal velocities—75 cm per second (larvae) and 7.5 cm per second (pollen).*

A standard Weather Service instrument shelter was placed at ground level and contained a hygrothermograph and a temperature sensor identical to those mounted on the tower booms. Solar insolation below the canopy was measured with a recording pyranometer fastened to the roof of the instrument shelter.

In 1974, all wind sensor data were recorded on strip-chart recorders running at 3.8 cm per minute; this speed was necessary to obtain a sufficiently detailed record of the fluctuations in the wind. Over 2,300 m of strip charts were digitized by hand to produce computer compatible punched-paper tapes containing the meteorological data. The digitization process proved to be an arduous task that required over 6 man-months to complete. In 1975, wind and temperature sensors were directly connected to a digital data acquisition system that measured the signal from each sensor once every 6 seconds. These measurements were recorded directly onto a nine-track magnetic tape in a computer-compatible format. The tapes were checked daily for validity to insure that the data acquisition system was operating properly. As a result, the meteorological data were ready for analysis almost immediately after the field experiment was completed. The data were analyzed in 10-minute intervals to fit the requirements of the dispersion model.

The Larval Samplers

The larval samplers consisted of a series of traps and were designed to fit the following specifications:

• The individual traps should be cylindrically shaped so that they would project the same area regardless of the direction of the wind.

• To allow air to pass through the trap 0.01 mesh hardware cloth covered with Tack-Trap® was used, otherwise the flow field in the vicinity of the trap might be disrupted and prevent the impaction of passively-blown larvae. At the same time, there was little chance that a larva trailing silk could pass through both sides of the cylinder without being caught.

• Traps would be placed at or above the top of the forest canopy because only those larvae that are in

that airspace have any potential to be carried any distance by the wind.

- The traps must be readily accessible because they must be lowered and checked for larvae at least 3 to 4 times a day.
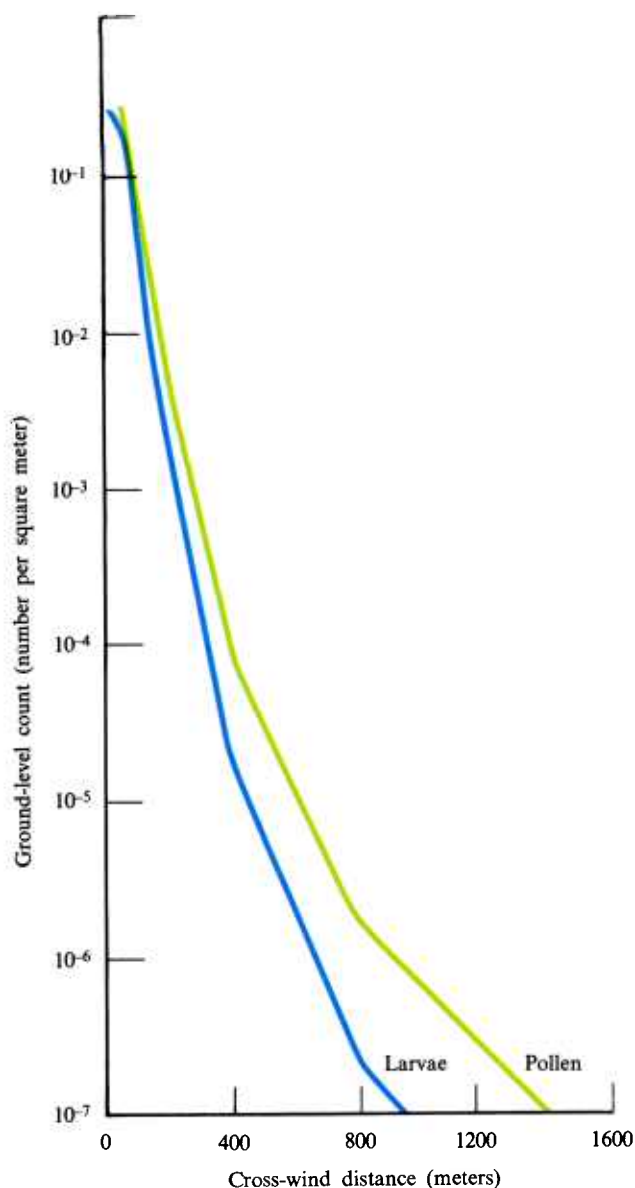
Figure 4–70.—*Corresponding crosswind ground-level deposition 160 m downwind from an elevated point source for a slightly unstable atmosphere and two terminal velocities—75 cm per second (larvae) and 7.5 cm per second (pollen).*

- Each sampler should project a total surface area capable of trapping from 50 to 300 larvae per hour at 0.16 km from their source. These figures were obtained from the model's predictions after considering the egg-mass density in the source area, the number of viable eggs per mass, a 20-day period of hatch, and 1 percent of the larvae dispersing per hour during that period.

In 1974, eight samplers, each containing eight 30×90 cm cylinders (4.4 m^2) were placed in a grid throughout the 0.65-km^2 study area. The traps were suspended on a system of pulleys from a cable between two large trees in four rows of two traps each. It required a crew of three people at least 30 minutes to check the traps and record the data at each sampling site.

In 1975, the samplers were redesigned to facilitate trap checking. A T-shaped unit was constructed (fig. 4–74) consisting of a guyed 15-m telescopic television mast with a 4-m cross boom and from which six traps were suspended (three on each side) on a system of pulleys. The cylinders were cut down to 30×60 cm so that they could be lowered more easily for checking and then raised to their original position (fig. 4–75).

Location of Samplers

The model's predictions suggested that dispersal is basically short range, with most of the larvae being deposited near the source. In the 1974 pilot study, the samplers were placed on a grid within the 0.65-km^2 study area.

In 1975, 21 samplers were set out in three concentric circles at 60, 120, and 180 m from a central circular source region 20 m in diameter, which had one sampler at its center (fig. 4–76). The orientation of the cross booms at all locations was tangent to the circle; the center sampler cross boom was aligned north-south. The samplers were staggered in azimuth on the successive circles to insure that at all times at least one sampler would be more or less downwind from the central source region to intercept the dispersing larvae regardless of wind direction. Each sampler had slightly more than 1 m^2 of collecting surface.

The sampler located at the center of the source region provided a means of estimating the entrainment rate, the only undetermined parameter that the model requires. All other requisite parameters, except for the dispersion coefficients, were weather related and were measured independently by the meteorological installation; values for the dispersion coefficients were obtained from the literature. Regardless of the wind direction, a centrally located sampler entrapped larvae from some portion of the source region. The number of larvae trapped was compared to the prediction of the model for an arbitrary entrainment rate; this value was then normalized to make the prediction equal to the observed number at the center trap.

At this point, the dispersion model can be run to predict the number of larvae trapped per square meter on a sampler located at a given distance from the center of the source region as a function of the diameter of the source. Figure 4–77 shows the results of this calculation for the source diameter eventually selected (20 m). Representative values for weather parameters are assumed to make these calculations. The entrainment rate is completely arbitrary at this point in discussion (as well as completely unknown). As an example, however, if the entrainment rate is such that 1,000 larvae are caught in the central trap during the sampling period, then the number of larvae reaching a ring of samplers at 200 m is expected to be small (1 or 2). Of course, observation of higher num-

Figure 4-71.—*Crown-level deposition for a 10 km²
region with an initial infestation of 60 larvae per
square meter.*

Figure 4-72.—*Meteorological tower 20 m high used
in model verification studies. Booms supporting
wind and temperature sensors are located at 20, 15,
and 10 m.*

bers would be expected in traps located at intermediate distances, as figure 4–77 shows. The entrainment rate is still not known but it is hoped that the duration of the sampling period can be regulated to obtain reasonable trap catches.

Source Population

In 1974, the natural gypsy moth population within the 0.65-km^2 study area was used; the egg-mass density varied from 200 to 1,500 per hectare.

After reviewing the results from the 1974 study, it was decided to conduct a large-scale point-source release in 1975 so that better control could be maintained over the numbers and original source of dispersing larvae. To estimate the number of eggs, the entrainment rate and the duration of the hatch period were estimated. At an entrainment rate of 1,000 larvae per minute from the source area, larvae would accumulate at the rate of 300 per hour at the center trap (fig. 4–77). Realistically, this peak rate would occur for only 4 hours a day over a 5-day period of hatch. Ap-

proximately 1.2 million eggs would be required to provide this entrainment rate. It was anticipated that larval emergence would be more synchronous than usual because all eggs were preconditioned under the same regime of temperature and relative humidity. Two releases were conducted—the first comprising 1,100,000 viable eggs after natural hatch occurred in May, and the second release (850,000 viable eggs) after emergence of the first group of eggs was completed. Egg masses collected from Pennsylvania were dehaired, and the viable eggs were then separated, counted, and placed in Saran® mesh packets. The packets, containing 7,000–20,000 viable eggs, were stapled onto a group of trees within the 20-m diameter source area. In both years, egg hatch was monitored in the general area so that it was known when larval emergence began and when peak emergence occurred.

Results of the 1974 Field Study

Egg eclosion began on May 13 and was essentially completed by May 19, although individuals from

Figure 4–73.—*An anemometer bivane to measure wind speed, azimuth, and elevation angles; a temperature sensor in a radiation shield is mounted on the same boom.*
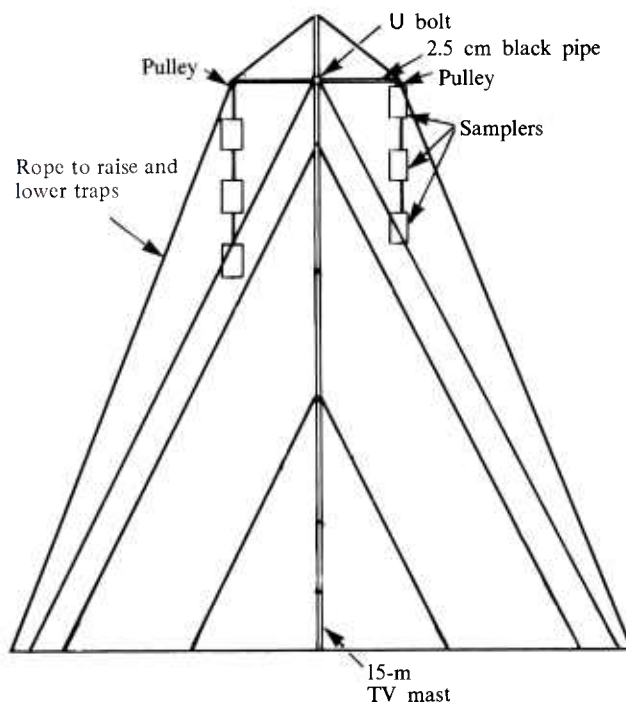
Figure 4–74.—*Schematic of 15-m high sampler.*

many egg masses emerged until the 25th (fig. 4–78). Peak emergence occurred over a 4-day period (May 15–19) when the daily average temperature exceeded 18° C. Larvae were first trapped on May 16 (fig. 4–78); on the basis of the numbers recovered, most dispersal in the area occurred over a 5-day period between May 18–22. Peak dispersal was synchronous with peak hatch, though there was a 2–3 day lag. This agrees with earlier observations by McManus (1973a) but does not support reports in the early literature (Burgess 1913, Collins 1915) that peak larval dispersal usually occurred about 2 weeks after hatch was first noted. Since larvae usually leave the egg mass within 24 hours of emergence (unless precipitation occurs or temperatures are below 7° C), climb trees, and elicit dispersal behavior, maximum dispersal should lag slightly behind peak emergence.

Weather played an important role in greatly constricting the duration of hatch and dispersal. It was anticipated that large numbers of larvae would be trapped for at least a week; however, the weather changed drastically on May 20. Morning temperature dropped below 4.5° C, and except for a 1-day respite, the weather for the next 5 days was characterized by average daily temperatures below 13° C, precipitation, and overcast skies. In short, dispersal was shut off by local weather.

There was a significant variation in the numbers of larvae trapped at the eight sampler locations—55 percent were trapped at two sites and reflected their close proximity to areas where the greatest number of egg masses were counted; however, the distribution of daily counts indicates that the maximum number of larvae were trapped on either May 18 or 19 at all eight

Figure 4–75.—*Ground view of sampler positioned in forest canopy.*

locations throughout the study area. The 1974 results were informative but not adequate to provide the necessary data to verify the predictions of the dispersion model. The source function (potential number of dispersing larvae) within the study area was extremely variable—this was verified by the unequal numbers of larvae that were trapped at the eight sampler locations. Because of this it was impossible to relate the source strength near any one sampler unit to the number of larvae trapped under a known set of meteorological conditions.

Results of 1975 Field Study

It became obvious that the only way to obtain a good measure of source strength was to create an infestation confined to a predetermined source region. Because of the control that could be exercised over egg quality and their subsequent environmental exposure, the duration of hatch would be shorter than that of eggs in the natural state. Consequently, two separate experiments involving two egg batches were planned: These would be conducted in the same total length of time as that of natural hatch and dispersal. In this way, only weather conditions associated with the natural dispersal period would be encountered while, at the same time, adverse weather could not hamper the whole experiment as in 1974. Both experiments were carried out as planned in 24 days. Representative results from the first experiment follow.

Egg packets containing 1,100,000 eggs were affixed to trees throughout the 20-m diameter source region. The first hatch was noted on May 18, and sampler counts began on May 20. Trap counts declined by May 25 concurrent with cessation of hatch. On the basis of counts of a representative egg packet, an estimated 82 percent of the total eggs hatched.

The data for the morning of May 23 are presented as an example of results and represent the peak dispersal period as determined by the central sampler trap counts. From 0900–1300 hours the wind blew primarily from the south, southwest; the 10-minute average speed and directions as measured at the tower site are given in table 4-43. At 0830 hours, a low (150 m) overcast blanketed the area, air temperature was 16.5° C, and the relative humidity was near 100 percent. By 1120 hours, the sky cleared and stayed clear throughout the remainder of the day. A maximum temperature of 25.5° C was recorded at 1240 hours.

The wind speed, wind direction, and turbulence level were used as input data to the dispersion model to predict the numbers of dispersing larvae and their directions of travel. The standard deviation of wind direction in table 4-43 was used to determine the level of turbulence or, equivalently, atmospheric stability. In this case, the fluctuations were representative of a moderately unstable atmosphere.

During the period 0900–1130 hours, 909 larvae were trapped at the center sampler. This number is used to estimate an entrainment rate for the source region of 985 larvae per minute, an average value applicable to the entire period. With this parameter evaluated, a prediction of the number of larvae
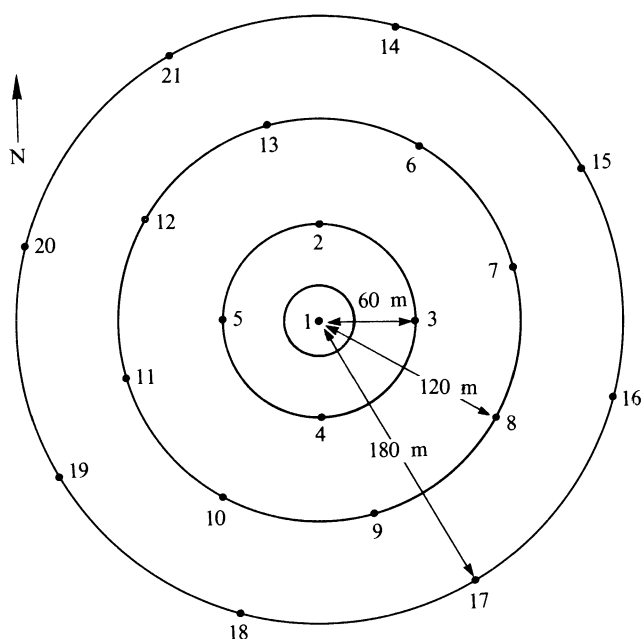
Figure 4-76.—Placement of an array of samplers for the 1975 field study. The 20-m diameter source region is shown in the center.

trapped at each sampler location during the morning period can be made. For a second dispersal period from 1130-1400 hours, 205 larvae were trapped at the center sampler. On the basis of this number, an entrainment rate of 260 larvae per minute is calculated for this period. The sampler-count prediction for this latter period combined with the corresponding morning values are given in fig. 4-79; the actual number of larvae trapped by each sampler are also given. For purposes of orientation, the mean wind directions are shown in the same figure.

An obvious conclusion readily apparent in this figure is that larvae are transported in the direction the

wind is blowing. With only one exception—and that being a single larva—all larvae trapped at 120 m or 180 m were downwind of the source. At 60 m, 90 percent of the trapped larvae were also in the downwind traps.

Figure 4-79 also shows that the model systematically underestimates the number of larvae trapped on the downwind samplers, with the largest underestimates usually at 60 m and decreasing for the more distant samplers. This result suggests significant reentrainment and subsequent redispersal of the larvae after their initial impacts, in accord with the random-walk concept previously discussed. This process creates a slowly changing source geometry as time progresses. On this occasion, the source region "migrates" toward the north and becomes broader because the wind direction remains relatively steady throughout the day. Support for this hypothesis is the observation that at a distance of 60 m most of the larvae were trapped in the afternoon, during the second dispersal period. The entrainment rate at the center sampler for this period was only 26 percent of that for the morning period, and far fewer larvae should have been trapped. As the migrating source moves closer to the 60 m downwind samplers, however, the number of larvae actually trapped in these samplers increases, as observed. The effects of source migration are mitigated with distance, and better agreement between observations and predictions would be expected as the distance from the source increases; the observations bear this expectation out. Eventually, reentrainment and redispersal must be worked into the model. Such a modification must await a comprehensive analysis of the 1975 dispersal data, and two additional parameters—the fraction of larvae redispersing and the average time interval between successive dispersal incidents—must be introduced.

Active larval reentrainment does not alter the nature of the dispersal process—it is still a short-range phenomenon. For example, during the 13 days of the 1975 experiment, the catch per sampler as a function of distance from the source was as follows: For the central sampler, 5,245 larvae were trapped; at 60 m, 142
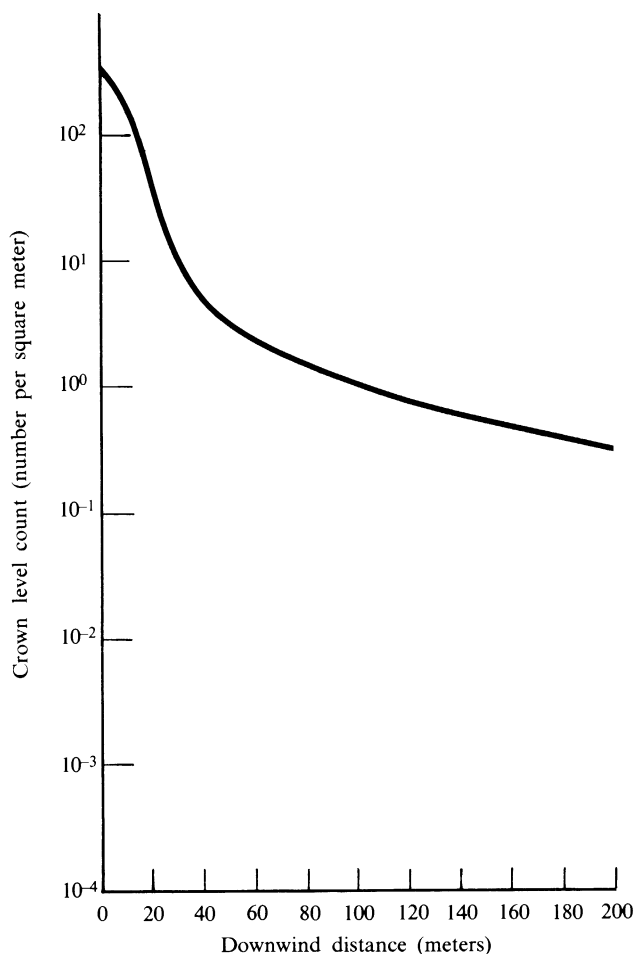
Figure 4-77.—*Crown-level deposition prediction for the 20-m diameter source region.*

larvae; at 120 m, 108 larvae; and at 180 m, 46 larvae. This result integrates overall wind directions, dispersion conditions, and redispersal events. Obviously, considerable deposition occurs near the source region, while the number of larvae trapped decreases very rapidly as the distance from the source increases. Secondly, a considerable amount of dispersion takes place below crown level. At a distance of 60 m almost one-third of the trapped larvae were caught in the bottom tier of traps; these traps were well into the canopy. Even in the source region, over half of the larvae trapped (2,613) were caught at the bottom level. Long-range transport is impossible for dispersion taking place in the canopy. Only those larvae that disperse above the crown are in a position to be transported long distances. Therefore, consideration should perhaps be limited to only the top tiers of traps on each sampler, traps that are above the crown level. Larvae trapped at this level escaped entanglement with elements of the

canopy (limbs, branches and twigs) and dispersed freely over the crown, and free dispersal is the exact condition that the model assumes to prevail.

For top-tier captures only, the model predictions should be reduced by a factor of three because only one-third of the total trap area is contained by the top tier. Also, recall that the entrainment rate is still a free parameter and can be adjusted to give the best fit between the observed and predicted trap counts. It is clear that compensating corrections can result if both these factors are taken into account; however, in view of the total uncertainty inherent in the dispersion model itself—a factor of three (as discussed previously)—both can be ignored. If the entrainment rates as estimated by the total trap counts at the center are thus regarded to be representative of the rates at which larvae are dispersing above the top of the canopy, then trap capture predictions can be compared to the actual catch at the top level (fig.
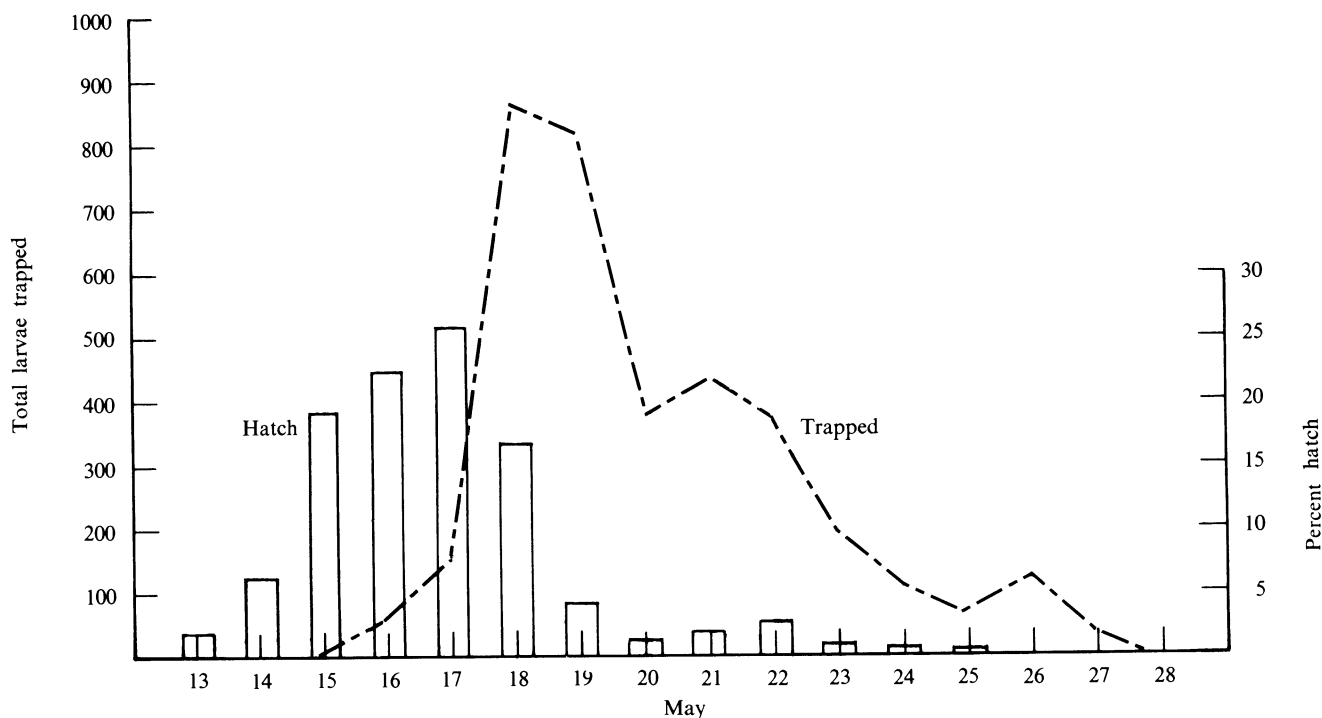
Figure 4–78.—*Total larvae trapped as related to egg hatch* (*Harwich, Mass., 1974*).

4-80). The agreement between the predictions and the observations is now excellent. Therefore, even for these larvae, dispersal is a short-range process.

In conclusion, the dispersion model is able to provide a measure of the entrainment rate that is reasonable and that also estimates sampler catches with a high degree of precision under the appropriate conditions. As previously noted, most dispersion models are good only to a factor of three, and this one should be no exception to that rule.

Modification of the Atmospheric Dispersion Model to Include Terrain Effects

The larval atmospheric dispersion model described earlier in this section can be modified to incorporate the effects of terrain features. Although the model that was developed and tested seems to accurately describe larval dispersal on relatively flat terrain, it is realized that topographic features can induce a nonzero-average vertical wind component. This vertical flow can pick up the larvae and disperse them over a greater distance; as an example, the hills and ridges of Pennsylvania may present such a situation.

Description of Modified Model

Terrain features generate wind fields in their vicinity either by directly interfering with the smooth flow of wind or by altering air-mass properties that in turn create local circulation patterns. Hills and ridges are typical obstructions that interfere with the airflow
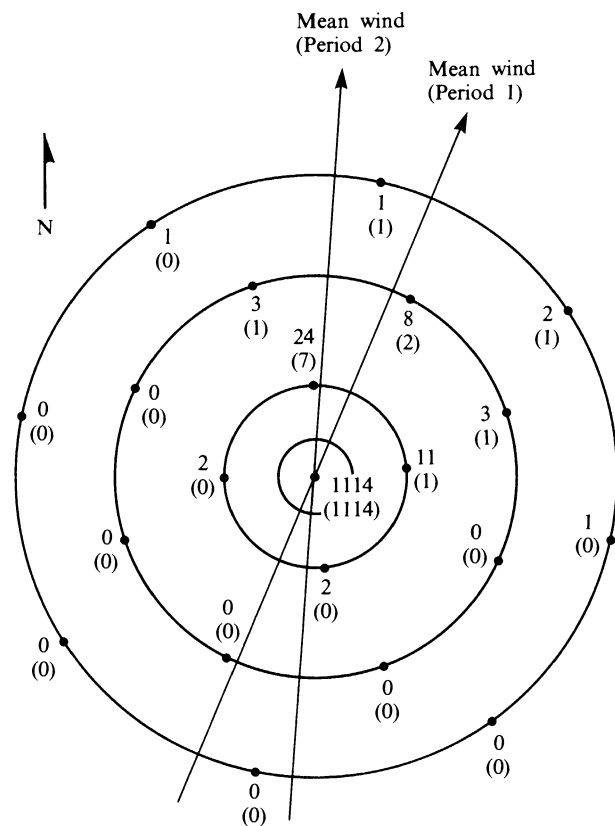
Figure 4-79.—*Comparison of sampler count prediction with the actual number of larvae trapped on 23 May 1975, between 0900 and 1400 hours.*
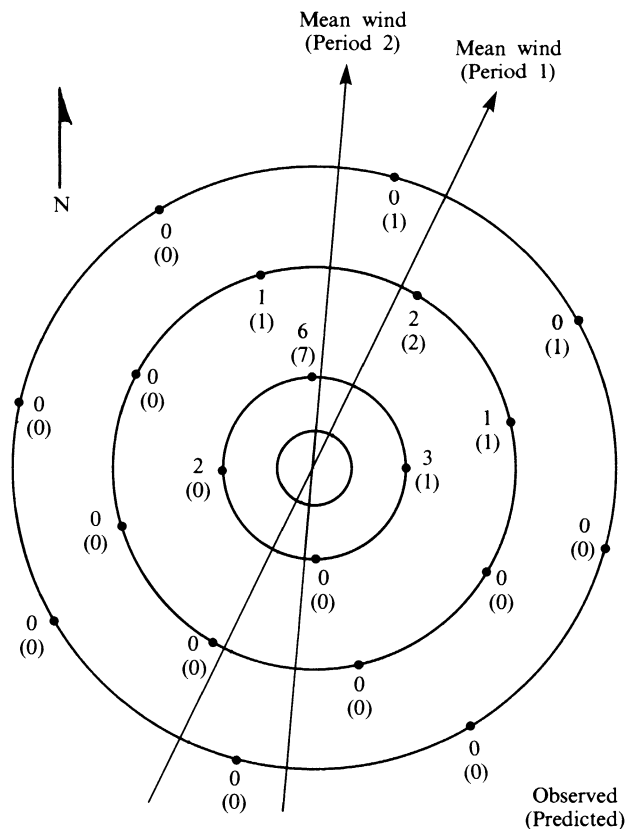
Figure 4-80.—*Comparison of top-tier (above canopy) count prediction with actual number of larvae trapped at this level on May 23, 1975, between 0900 and 1400 hours.*

Table 4-43.—*Ten-minute average wind speed and direction, May 23, 1975*

Time	Wind speed (m/second)	Wind direction (degrees)	Standard deviation wind direction (degrees)
0900	2.5	213	16
0910	3.3	221	12
0920	2.4	219	19
0930	3.0	221	15
0940	1.9	225	18
0950	2.0	223	19
1000	2.0	214	21
1010	2.2	218	16
1020	2.4	203	18
1030	2.4	211	20
1040	2.5	198	21
1050	2.0	194	32
1100	2.2	193	25
1110	2.8	172	20
1120	2.5	173	19
1130	2.7	174	17
1140	3.6	180	16
1150	3.3	181	18
1200	3.3	183	21
1210	3.2	186	13
1220	2.9	187	22
1230	3.0	178	16
1240	3.7	176	18
1250	4.3	174	15
1300	3.9	185	22
1310	4.6	185	13
1320	5.3	185	16
1330	4.5	195	21
1340	4.4	193	22
1350	4.9	209	16

on the surface of the earth; these features force a flow of air over and around them, not only changing the mean flow but generating mechanical turbulence as well. The "sea breeze," a meso-scale convective flow resulting from the land/sea temperature differential in the presence of large bodies of water, is an example of an alteration of air-mass properties. Both situations can be important to larval dispersal: The hills and ridges of eastern and central Pennsylvania seem to form natural foci for high population densities; the distribution of populations along the coast of New Jersey is apparently affected by the sea breeze.

In the simpler version of the model as given in equation 2, the wind speed and direction are regarded as not changing with time or location for a given

interval of time. As a result, the puff centers are in uniform motion along the x-axis for that given time interval. However, the wind speed and direction do depend upon time and location. In the one-dimensional case, $U = U(x, T)$ where $T = (t - t')$ can be written to emphasize this dependence. Then the puff trajectory becomes:

$$X(x, T) = \int U(x, T) \, dT$$

In other words, the factor $U(t - t')$ can be replaced in equation 2 with X as given by this last equation. The generalization of this procedure to three dimensions yields the basic equation for the modified model:

4. $(2\pi)^{3/2} C_0(x, y, z, T) = \dfrac{1}{\sigma_x \sigma_y \sigma_z} \exp$

$$- \left\{ \frac{1}{2} \left[\left(\frac{x - X}{\sigma_x} \right)^2 + \left(\frac{y - Y}{\sigma_y} \right)^2 + \left(\frac{z - Z}{\sigma_z} \right)^2 \right] \right\}$$

where the three equations

5. $\qquad X(x, y, z, T) = \int U(x, y, z, T) \, dT$

$\qquad Y(x, y, z, T) = \int V(x, y, z, T) \, dT$

$\qquad Z(x, y, z, T) = \int W(x, y, z, T) \, dT$

are the three parametric equations that specify the three-dimensional trajectory of the puff in time and space; U, V, and W are the along wind, crosswind, and vertical-wind components of the wind field as they depend upon location and time. The larval settling velocity is incorporated in the W-term. The larval concentration at the point (x, y, z) and at any given time t owing to an instantaneous release $Q(t')$ at

the origin at time t', is still given by equation 3. The model algorithm divides the integration integral of equation 3 into a series of subintervals in each of which the entrainment rate Q is constant. In essence, the problem is reduced to that of finding the puff trajectory, as determined by the local wind field and its fluctuations, substituting this information via the parametric equation 5 into equation 4 and performing the indicated integration of equation 3.

Input variables are:

- Source location and dimension.
- Sampling point location.
- Dispersion time period.
- Larval entrainment rate (as a function of time).
- Atmospheric stability classification.
- Larval settling velocity.
- Wind field associated with selected terrain feature (as a function of time).

The wind-field input data are formatted as a time series of three three-dimensional $10 \times 10 \times 10$ grids. The grids form orthogonal coordinate systems referenced to the terrain features. Each system contains the values for one component of the wind vector as it is distributed in space and remains valid until temporal changes force its modification. The gridded wind-field points must be obtained from either direct in situ measurement or theoretical calculations based on a wind-field model for the case under investigation. Finally, simply because pertinent meteorological data are not available, the implicit assumption must be made that atmospheric stability is the same over the entire region modeled. One would generally not expect this to be the case; for example, turbulent intensity at the tops of ridges or just below the tops on the lee sides might be expected to be greater than that in the valley below. However, the model can be modified to allow the inclusion of spatially varying atmospheric stability—the turbulence field—if these data become available subsequently.

The resultant model allows the formation of theoretical predictions on larval dispersal patterns from the mathematical descriptions of the wind fields associated with terrain features. Output variables are:

- Sampling-point location.
- Larval concentration (number per unit volume) in the air at the sampling point.
- Horizontal trap count (number per unit area) at the sampling point.
- Vertical trap count (number per unit area) at the sampling point.

The model can simulate dispersion from point sources and extended sources. For this latter case, the source region must have sides of the same length—that is, it must be square. Complicated source region geometries are treated as a superposition of square sources configured to approximate the outlines of the actual source region.

Larval Dispersion in the Vicinity of Selected Terrain Features

Sea Breeze Dispersion

The sea breeze is a well-understood phenomenon; its relative simplicity is adaptable to mathematical modeling (Wilson 1967). The fully developed sea breeze prevails in the early afternoon on days characterized by weak gradient flows. The sea breeze is characterized by a convergence frontal zone some 1–2 km in extent located some 10–20 km inland (fig. 4–81). As the onshore wind approaches the convergence zone, its horizontal velocity is reduced (ultimately to zero at the front), while the vertical velocity increases. The associated offshore wind—the absolute velocity of which is always less than that of the onshore wind—shows a similar dependence on distance. Updrafts of about 100 cm per second are computed for the convergence zone well above ground level (Lyons 1975); at crown level, the updrafts are not as strong but still may average about 20 cm per second. The implications of such a wind-field pattern for larval dispersal are clear: The opposing windflows (that is, inwardly converging winds) focus the dispersing larvae in a narrow band parallel to the coastline; the prevailing updrafts may extend the normally short-range dispersal process and bring into this

region larvae from a larger surrounding area. These two mechanisms can lead to heavily infested areas, or "hot spots," that extend along the coast.

To model the dispersion near the coast, a uniformly infested source region 10 km² and situated on a level coastal plain is considered. That side of the region nearest the coast runs parallel to and is 5 km inland from it; the wind-field convergence zone is situated 5 km further inland from this edge. The sea breeze characteristics described above are included in the wind-field input grid. The 75 cm-per-second terminal velocity of 1-day-old larvae is subtracted from the magnitude of the vertical wind component. Simply for convenience, any along-shore wind component is not included. To run the dispersion model, the source region is divided into 100 1-km² areas with the spatial dependence of the mean winds given by the wind-field model. A dispersion time interval of 1 hour during the early afternoon when the wind-field model is valid is selected; atmospheric stability is taken as moderately unstable everywhere in the source region.

The predicted areal density (number of larvae per square meter) on a horizontal surface at crown level, along a strip perpendicular to the coastline and extending from one edge of the source region to the other, is plotted in figure 4-82. As this figure shows, the model predicts a band of enhanced infestation parallel to the shoreline. As expected, the primary cause for its formation is the convergent circulation pattern of opposing windflows. However, although

persistent updrafts exist in the convergence zone, they alone are not of sufficient magnitude (especially at crown level) to extend the dispersal range significantly.

For purposes of comparison, figure 4-83 maps the observed dispersion patterns near the coast in New Jersey. It is interesting to note the development of these patterns, particularly in Cape May and Ocean Counties. For example, only single isolated populations were mapped in 1970 (fig. 4-83, A). By 1973, however, a substantial area is severely defoliated in a narrow band parallel to and at some distance from the shore (fig. 4-83, B). Finally, in 1975, (fig. 4-83, C) fragmented remnants of this population are distributed in a band along the coast. Through successive years, a well-marked band of heavy defoliation 2-3 km in width developed about 8-10 km inland. The spatial characteristics of this band (namely, its extent and location) as well as its relative stability from year to year after establishment indicate that it is possibly a manifestation of the sea breeze.

The model does not show the rapid along-shore spread simply because the along-shore wind component is set equal to zero. Actually, as the sea breeze develops, and particularly if it persists, it is acted on by the Coriolis force, which, in the Northern hemisphere, causes a wind veer to the right. Therefore, a westerly flowing onshore breeze swings slowly north in this example, while simultaneously the offshore wind

Figure 4-81.—Sea breeze wind field. The infested area is symmetrically located along the surface boundary of the convergence zone.
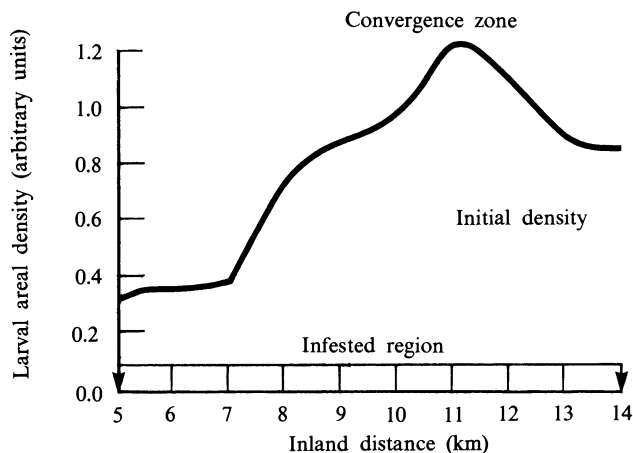
Figure 4-82.—Larval deposition for the sea breeze wind field depicted in figure 4-81.

turns south. Such a flow regime gives rise to a strong region of wind shear in the convergence zone, enhancing turbulence there as well as producing winds that have along-shore components of flow. These components serve to blow the dispersing larvae in the north-south direction, and at the same time they are blown toward the convergence zone. Given an initial "point" source, the above process leads quickly to the formation of an extended band of enhanced infestation parallel to the coast, as is observed. For the initial source configuration studied, model predictions are in excellent agreement with the observations. However, if suitable meteorological data showing the wind shift for the sea breeze become available, the model can be validated.

Ridge Flow Dispersion

To date, a consistent and useful data set and/ or model of the wind field in the vicinity of a hill or ridge has not been located. This result is not entirely surprising, because the flow regime about such an obstacle is extremely complicated and a function of many parameters, the most notable among these being the angle at which the wind intercepts the obstacle. For example, in the case of parallel-ridge flow, two entirely different wind fields are found if the wind in the first instance is across the ridges or, in the second, parallel to them. Intermediate angles lead to a partial channeling of the flow between the ridges while the remainder of the air flows over the top. In addition, the measurement of these wind fields as well as their subsequent specification for a given set of meteorological conditions is not easily attained, especially for what might be called the "general case." However, a simplified treatment to test the model is possible and can elucidate the nature of the dispersion process in the vicinity of ridges.

An indication of the type of wind field associated with flow over rough terrain is given by Start et al. (1975). Secondary flow patterns are induced by obstacles protruding into the primary flow pattern; wake turbulence extends downwind from these obstacles and often a region of enhanced turbulence and reduced wind speed can be created. Green (1976) suggested that interference with the primary flow by

obstacles with characteristic length L produces secondary flow patterns with similar length scales, and with wind speeds approximatley 15 to 20 percent of that of the primary flow. In particular, windflow at an angle to two parallel ridges will induce a helical rotor with dimensions comparable to the ridge spacing and height. Such a construct is in accord with the cavity-flow picture of Start et al. (1975).

Accordingly, a wind-field model for windflow aross parallel ridges was developed using these qualitative ideas. Reference to topographic maps of central Pennsylvania established that a great portion of the terrain consists of a series of gently curving parallel ridges rising some 425 to 460 m above their valley floors. A representative sample of the terrain was selected near Williamsport, Pa. Here the ridges are about 3.2 km apart and rise approximately 365 m above the valley floor (fig. 4–84). An elliptical rotor with major axis equal to the ridge separation and minor axis equal to the ridge height is assumed to exist in the region between the two ridges; the wind speed of the rotor is taken as 20 percent of the free airflow above the ridge. The along-valley flow component of the wind is not modeled but instead is set equal to zero.

For ridge flow, the dispersion is modeled from an infested 500-m square area located on top of the upwind ridge (fig. 4–84). This situation might correspond to the case where a small "seed" population has become established on the ridgetop; the predicted dispersion pattern delineates the probable areas of subsequent infestation. Alternately, one can regard this single source area as only one portion of an infestation that runs along the entire ridgetop; the dispersal pattern for this instance can be constructed by simply superimposing the single source patterns displaced laterally by distances equal to the lateral displacement (from some arbitrary reference) of the elemental source areas corresponding to them. As in the sea-breeze model, the source area is assumed to be uniformly infested; a dispersion time interval of 1 hour is considered for which the cited wind field model is valid; and a moderately unstable atmosphere prevails. As usual, a larval settling velocity of 75 cm per second is used.

Map A

NEW JERSEY STATE DEPARTMENT OF HEALTH
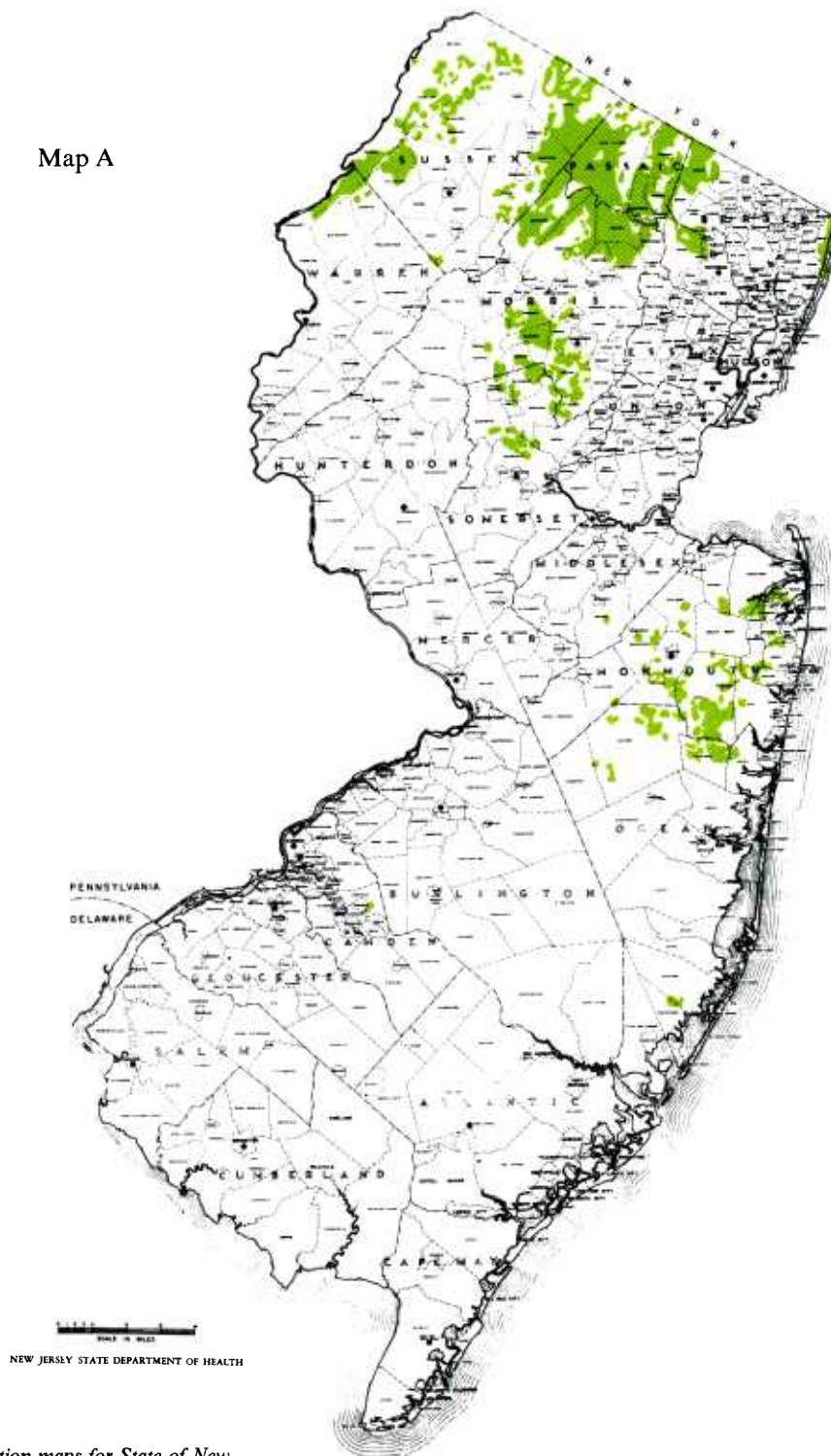
Figure 4–83.—*Defoliation maps for State of New Jersey, showing the progression of defoliation along the coastline: A, 1970; B, 1973; C, 1974.*

Map B

NEW JERSEY STATE DEPARTMENT OF HEALTH

Map C

NEW JERSEY STATE DEPARTMENT OF HEALTH
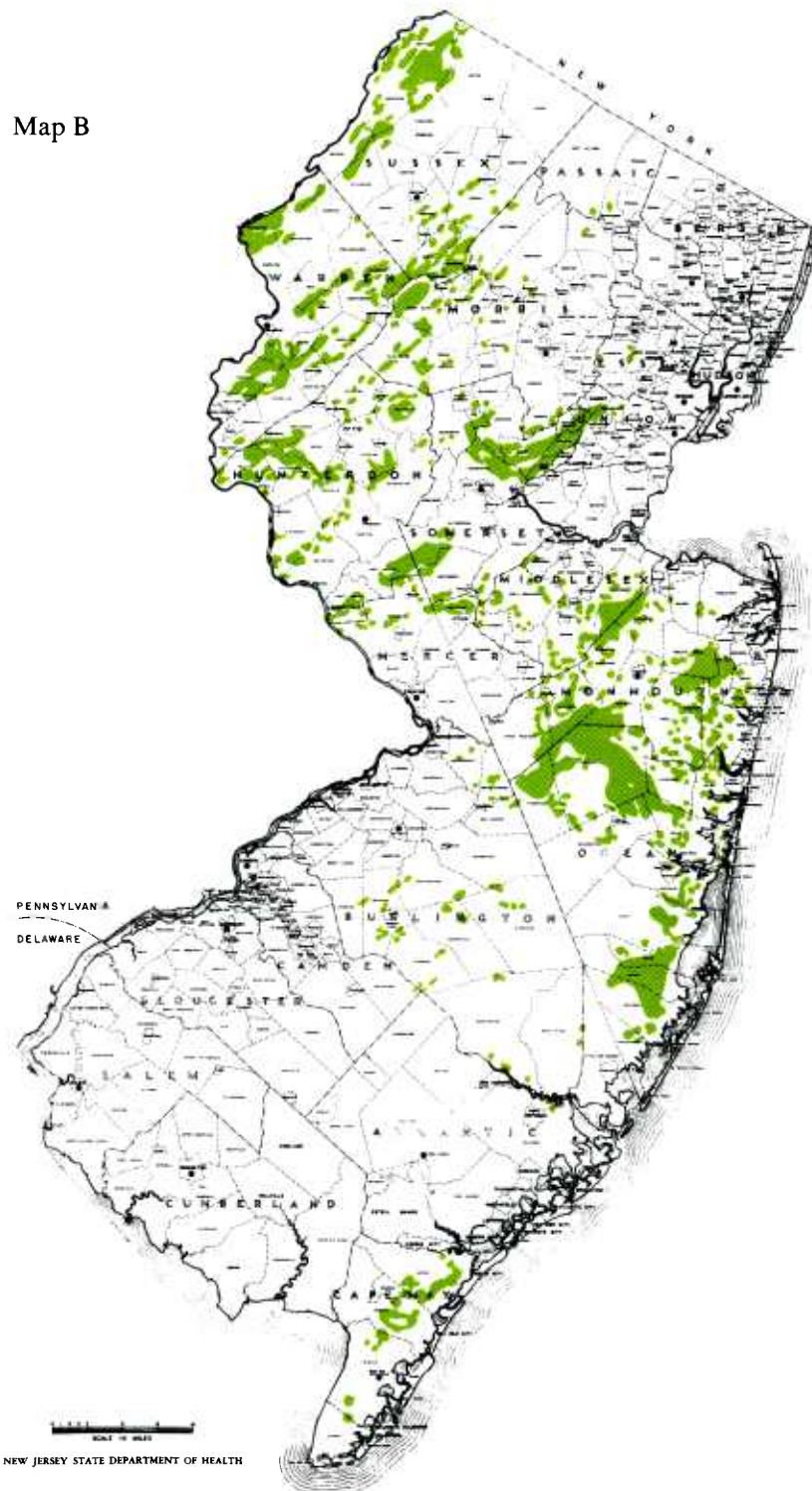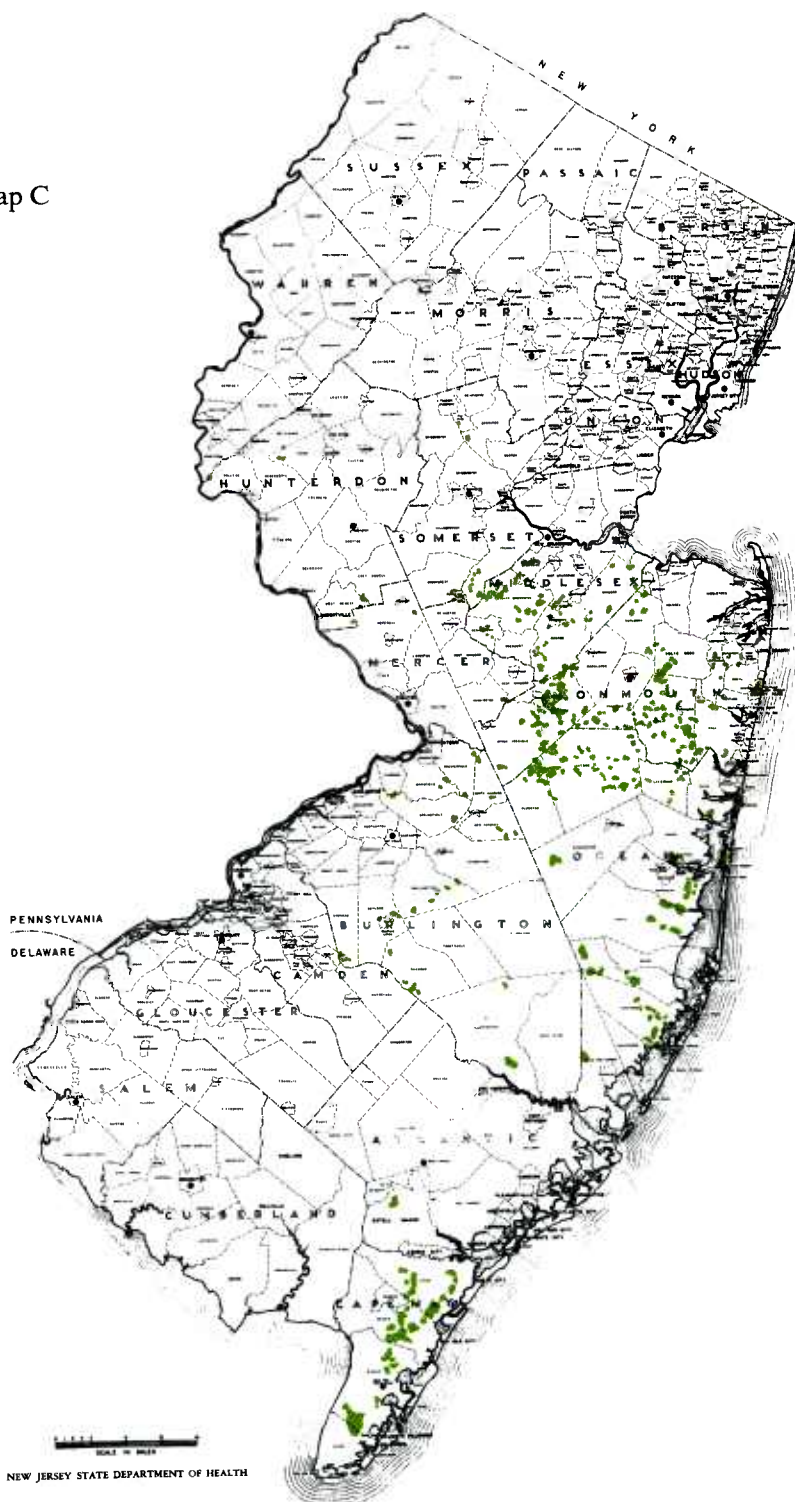
The predicted crown-level deposition isopleths in figure 4–85 are normalized to unity at the point of maximum deposition. Only one-half of the dispersion pattern is shown; the pattern is symmetrical about a line through the source area and perpendicular to the line of ridges. The deposition isopleths delineate the dispersion pattern to be expected for the terrain and wind field modeled. Note that the maximum deposition occurs 3 km directly downwind of the source region. Moreover, approximately 75 percent of the larvae are deposited in the area (fig. 4–85) (including the second half of the deposition pattern not shown). Owing to the strong updrafts in the vicinity of the source ridge, the larvae are initially lofted up from the crown in the source region, a lifting that continues (but diminishes) as they traverse the valley until they encounter the downdraft region near the second ridge. As these downdrafts increase in magnitude, the larvae are brought down to the crown near the peak of this ridge. Ground-level deposition near the source region is also minimized by the drop in elevation from the ridgetop to the valley floor; in effect, the ridgetop
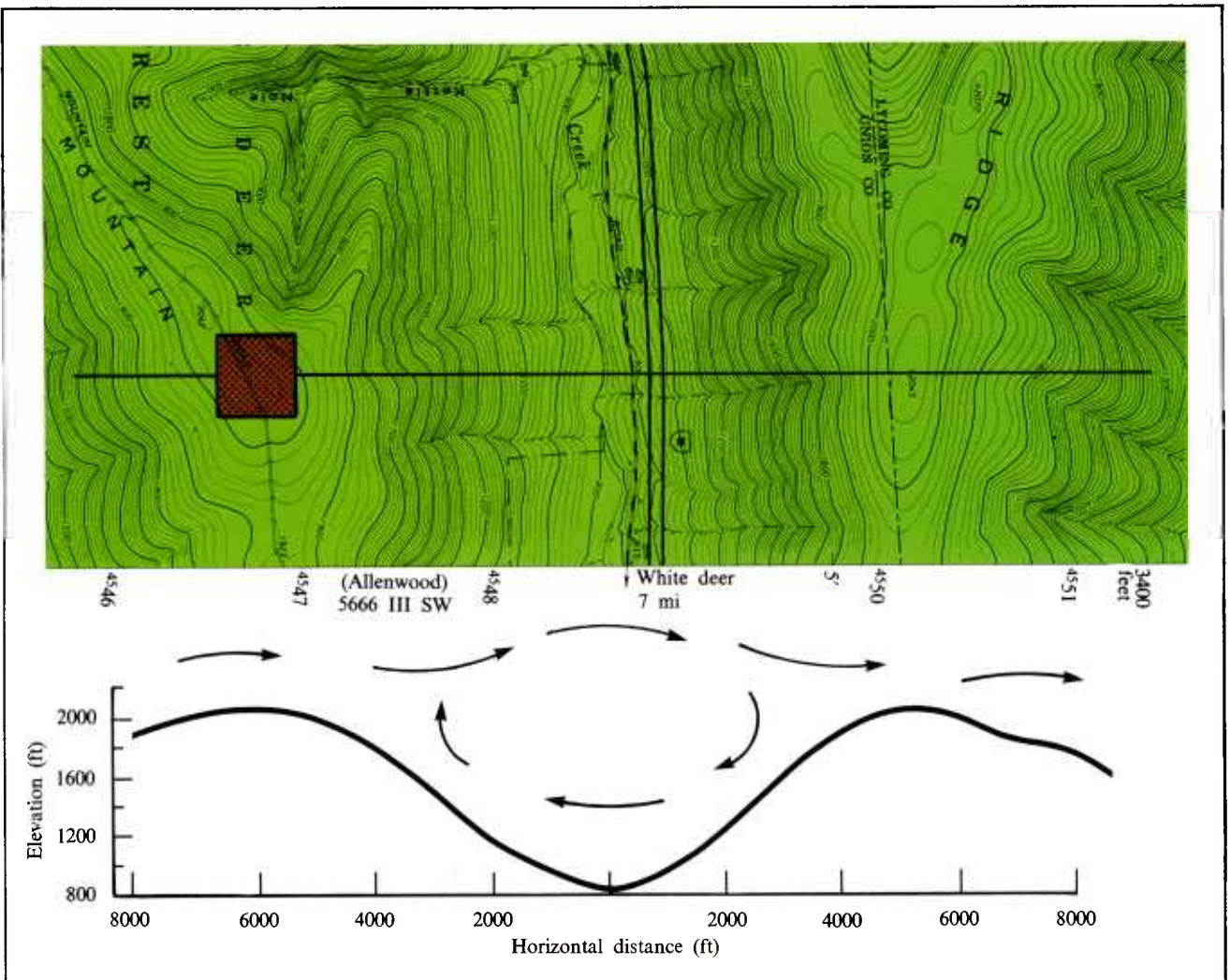
Figure 4–84.—*Pennsylvania ridge terrain and corresponding ridge-flow circulation.*

becomes an elevated source for which the maximum deposition is always found at some point downwind, rather than at the base of the source itself. These two factors—the source elevation and the updraft/down draft structure (induced by the terrain)—determine the nature of the deposition pattern.

As in the sea-breeze case, along-valley flow is neglected here. The result of such flow is easily visualized: It skews the deposition pattern in the direction to which the wind is blowing and causes an elongation of the deposition isopleths. Also, if the source ridgetop is infested along its entire length, a parallel band of maximum deposition develops near the top of the second ridge. As indicated previously, this result can be obtained by superposition of the deposition pattern shown.

With regard to observed dispersion patterns in hilly terrain, the most notable feature is the localization of heavy infestations at the tops of ridges. Infestations appear to spread from ridgetop to ridgetop. Aerial observations of defoliation patterns in central Pennsylvania during the summer of 1976 corroborated the above observation. Although some defoliation was visible almost everywhere in an infested area, valleys and lowlands generally were not as heavily defoliated as the ridgetops. However, in the vicinity of gaps and cuts in the ridge patterns, extensive defoliation from the ridgetop to the valley floor was apparent in several instances. Thus, actual dispersion involves "hops" from ridgetop to ridgetop, and the model results tend to verify this. It is hoped that it will be possible to obtain onsite ridge-flow data so that the model can be verified. How long the assumed flow structure prevails and how it becomes established are two important questions that need to be answered if the magnitude of dispersal in mountainous areas is to be understood.

Summary of Results

An advecting Gaussian puff dispersion model has been modified to simulate the effects of terrain features on the dispersal of newly hatched gypsy moth larvae. The model's predictions appear to explain the observed larval distributions for the sea-breeze and

ridge situations, even though simple assumptions were made about the nature of the wind fields.

The model successfully predicts dispersion patterns in two very different situations without a change in its major parameters and, most notably, without a change in larval settling velocity. As seen in the previous discussion, in the absence of updrafts the dispersion of larvae is a short-range process. For the sea breeze, the converging wind field plays the dominant role in the larval dispersion process, which still remains a short-range process because of the absence of strong updrafts. However, for the ridge-flow case, a significant extension of the dispersal range occurs as a result of the strong and persistent updrafts as well as of the variation in the height of the terrain.

Discussion

The initial objective of this study was to develop an atmospheric dispersion model that could be used to forecast the extent of dispersal (for gypsy moth larvae) and then to test the accuracy of the model's prediction in a natural forest situation. The secondary objective was to gain a better understanding of the processes
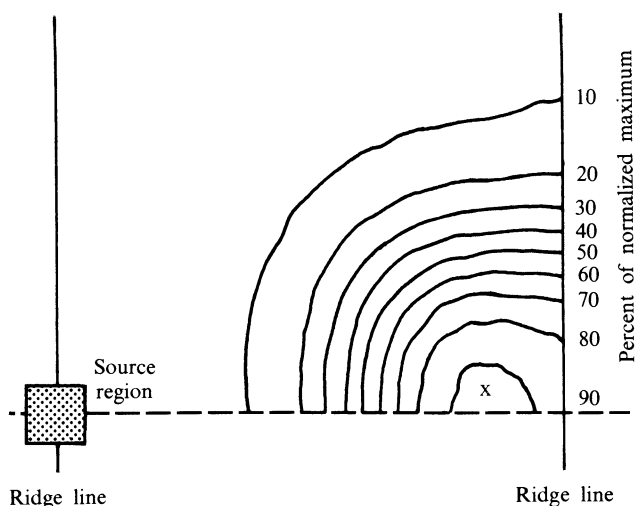
Figure 4-85.— *Deposition isopleths for the ridge-flow wind field of figure 4-84. The deposition isopleths (contour lines of equal deposition) are plotted to scale on the terrain map; the intensity of the deposition associated with a given line is expressed as a percentage of the maximum deposition that occurs directly downwind from the source (marked by x).*

involved and to identify areas where additional research is needed. One might question the value of being able to predict dispersal, especially if it cannot be prevented.

If an integrated pest-management system for the gypsy moth is implemented, it should employ a regional approach where decisions on individual land parcels are made in light of the status of the pest insect in adjacent areas. Therefore, the presence and potential influx of dispersing larvae from adjacent stands must be considered. On the basis of the modeling efforts, some numerical values can be placed on the probability of influx from surrounding areas if the extent of the source population and the topography of the land is known. Unfortunately, the technology is not available to predict the weather during the period of emergence and dispersal in spring; however, if this were possible, it would serve only to place limits on the magnitude or risk of dispersal beyond that which can now be predicted.

The empirical data supported the model's prediction that on relatively flat terrain larval dispersal is short range; stated in another way, the probability is high that most larvae will be deposited within 1 km of their origin. This does not exclude the fact that some larvae may be carried farther away.
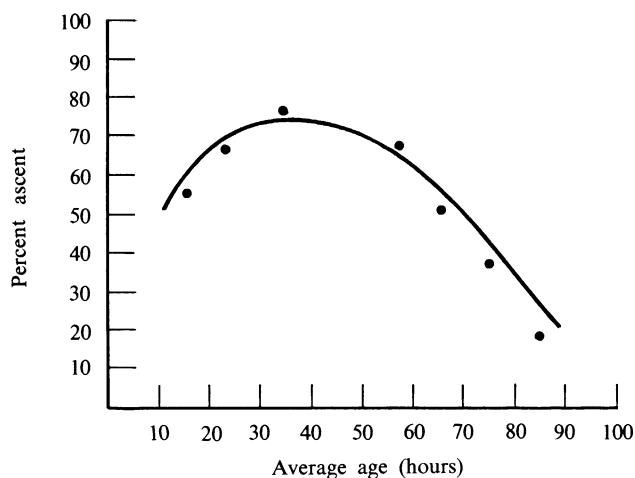
Figure 4–86.—*The effect of starvation (in hours) on the relative activity (measured as the percent that climb in response to light) of first-instar larvae.*

However, it is highly improbable that a large number of larvae will be picked up en masse and deposited in distant locations, resulting in isolated outbreaks. Examples of this can be found in the entomological literature—mass flights of female spruce budworm moths, grasshopper migrations, and long-distance movements of leafhoppers. In each of these cases, however, winged adult insects were involved, and certain orderly meteorological patterns were implicated such as low-level jet winds or frontal systems. To the best of our knowledge, there are no comparable examples in the literature that involve passively blown species.

Aside from the field data, certain realities support the conclusion that most dispersal is short range.

• Remote infestations similar to those that have been apparently eradicated by the Animal and Plant Health Inspection Service in Palos Park, Ill., in 1976 and in San Jose, Calif., in 1977 were found to encompass relatively small areas, despite the fact that these infestations existed for 2 to 4 years. In each case, intensive trapping surveys revealed that the infestations were confined to a 2.6 to 5 km² area. This suggests that dispersal over that period of years was not significant and that survival was very low, even in the absence of the natural enemy complex usually associated with the insect.

• Experienced gypsy moth scouts have often encountered so-called "wolf trees" in the woods—usually a large-diameter (d.b.h.) branching white oak that harbors hundreds of gypsy moth egg masses, while few egg masses are found on surrounding trees. Why does an aggregated distribution of the insect occur and persist on a single tree for a number of seasons? Perhaps many larvae dispersing from this point source get hung up by their own silk threads on many large branches of the same tree; the residual population on this tree has a high survival rate because there are many bark flaps and other natural resting places for the larvae. Those larvae that disperse and are scattered in the adjacent area may never establish on acceptable hosts or have a higher

probability of being attacked by parasites or predators. If dispersal were extensive, one might expect to find a "hot spot" developing from individual wolf trees, but this has not been the case.

• If dispersal is more extensive (as some have claimed), the gypsy moth would now be established farther to the south and west than is reported, despite the presence of prevailing winds. The point to be made here is that passive dispersal is costly to a species and is probably associated with high mortality. If the converse were true, the gypsy moth would now be distributed over a much larger geographical area, and remote infestations would be both more numerous and more extensive when finally detected.

Current observations suggest that dispersal is a very complex event; the many factors involved have been identified and are now better understood and it is recognized that many questions remain to be answered. For example, although most larvae disperse over a relatively short distance, an individual larva probably covers this distance through a series of short hops or several dispersal episodes. The question arises, when does an active larva cease dispersing and initiate feeding? Does it make a difference if a dispersing larva lands on a favored host (oak) as opposed to a nonfavored host (red maple)?

McManus (1973a) viewed dispersal as an innate tendency that larvae must satisfy before they initiate feeding. Active larvae always passed over fresh foliage of preferred species and instead dispersed from the tree rather than feed. Anyone who has reared gypsy moth larvae in the laboratory on either artificial diet in petri dishes or oak seedlings has observed the same behavior. When newly hatched larvae are confined in petri dishes with diet, they continually crawl around incessantly and trail silk but do not feed. This behavior usually persists during daylight hours, but by the next morning all larvae will have fed and are no longer active. The same turnoff mechanism probably occurs in natural populations, although other factors may also affect initiation of feeding. At some point, dispersing larvae expend their energy reserves or are no longer capable of producing silk; this may influence the change from dispersal to feeding.

Starvation is probably another determinant of dispersability in individual larvae. Laboratory simulation of dispersal from oak seedlings was conducted in 1973 in order to evaluate the effect of larval age on their willingness and ability to disperse (McManus 1972). Results indicated that larvae less than 15 hours old were reluctant to climb the seedling; larvae between 15 and 60 hours were the most active; and the vigor of larvae over 60 hours old declined rapidly (fig. 4-86). This agrees with Capinera and Barbosa (1976), who found that starved larvae survived between 48 and 60 hours but became inactive long before they died.

Consideration of these data is important because it provides insight into the probability of dispersal under natural situations. If larvae hatch but then are confined to the egg mass for more than 1 or 2 days because of adverse weather conditions, they become less active even though they are in a starved condition. These individuals, if still capable of actively climbing trees, are probably more likely to feed than to disperse. In essence, the incidence of dispersal within a given area will be shut down or at least greatly reduced because of local weather conditions. This situation occurred on Cape Cod in 1974.

There is apparent disagreement about the importance of wind velocity to the incidence of larval dispersal. A consensus of opinion is that the greater the winds, the greater the amount and extent of dispersal that can be expected. However, in laboratory tests (McManus 1972), when forced dispersal of larvae from oak seedlings was attempted, the number dispersed actually declined when the air velocity exceeded 6.7 m per second. When exposed to velocities above 6.7 m per second, larvae tenaciously hung on to the foliage and did not move. Those that were blown off did not release themselves on silken threads but were physically disengaged. Since the silk greatly affects the terminal velocity of the larvae, they would settle out sooner despite the greater horizontal winds. These observations agree with Richter (1970) who stated that aeronaut spiders exhibit most dispersal behavior when wind speeds were between 0.35 and 1.70 m per second. When the velocity exceeds 3 m per second, aeronautic behavior virtually stopped, and the spiders crept along the surface or hid to avoid the air currents. Jensen and Wallin (1965)

also noted that wind speed appears to be very poorly correlated with aerial aphid density. Wind speeds in excess of 2.2 m per second prevented aphids from alighting for approximately 24 hours. These examples suggest that passively blown species such as the gypsy moth are more stimulated to disperse by slight winds and that dispersal is actually inhibited by very strong gusty winds.

Dispersal is an important process in the population dynamics of the gypsy moth and is still a subject of much controversy. Earlier in this chapter, Campbell concluded that dispersal processes are important in the maintenance of an areawide outbreak, and these studies fully concur with this notion. Larval dispersal provides for a genetic mixing of individuals within subpopulations and assures a thorough redistribution of individuals over a sizable area. This would seem to provide at least two benefits to the gypsy moth: It prevents a deterioration of population quality or vigor in any one location, and it apportions the larvae throughout the food resource and at least temporarily reduces the chance of overcrowding and starvation in early instars. By the time the larvae reach the stage where severe competition occurs, they are both more mobile and capable of moving on to less preferred hosts, thereby increasing their chances of survival.

Mortality due to dispersal is probably high but has not been quantified. Even when newly hatched larvae are reared under optimal conditions on artificial diet, a proportion of the population does not initiate feeding and survive. In natural situations, many larvae will be deposited in places where they cannot survive (water or pastures), while others will end up on either unfavorable hosts (ash) or favorable hosts that are (for one reason or another) unacceptable. Each dispersal episode uses energy reserves and one can therefore assume that many unfed larvae will eventually encounter acceptable hosts when they (larvae) are in a poor physiological state; this also may be detrimental to their survival. All of the conditions discussed above must be considered when evaluating the role of dispersal in the dynamics of the gypsy moth. Certainly the geographical area of contiguous forest and the proportion of preferred hosts present will greatly influence the survival of dispersing larvae.

Defoliation maps, although after the fact, provide a collective overview of how outbreaks develop and progress from spot infestations to regional phenomena. Superimposed on these maps is the probable role of larval dispersal. Anderson and Gould (1974), through a series of defoliation maps, provided an excellent overview of the development and decline of an outbreak in the State of Connecticut during 1969–74. Clearly, the outbreak did not arise from a single focus or hot spot. Defoliation patterns suggest that within the State, a number of foci developed within a 1- to 2-year period, expanded, and then coalesced. By comparing the intensity of defoliation in any 2 successive years, it becomes obvious that the larval populations causing the defoliation are continually shifting—the larval dispersal and redistribution process is probably the one key that can account for this pattern. Anderson and Gould (1974) discussed the importance of larval dispersal to this outbreak and stated that a trend in defoliation occurred toward the north and east in the direction of prevailing winds; however, visual observation reveals that expansion of areas of defoliation intensity occurred in all directions. A comparison of defoliation overlays in successive years has produced similar patterns in Massachusetts and Pennsylvania.

It is concluded that dispersal is an innate behavior that all gypsy moth larvae exhibit; it makes no difference whether large or small larvae are more active, because although activity may be related to repeated dispersal episodes by individuals, it probably has little effect on the total distance covered by any one larva. Based on their mass and settling velocities, the differences between large and small larvae are negligible; length of silk alone has more effect on the settling velocity of the larvae.

The distance that larvae may be passively blown is determined exclusively by the physical environment, specifically vertical and horizontal wind fields. Strong horizontal winds alone will not result in long-distance dispersal—the vertical component of the wind (turbulence or vertical updrafts) must lift the larvae as they are carried in the direction of the horizontal wind.

Although larval dispersal does result in spread in the direction of the mean winds, one should not lose sight of the randomness associated with this process.

The concept of prevailing winds was discussed earlier in this chapter; the important point to remember is that the velocity and direction of winds are extremely variable within the period of eclosion and dispersal. Dispersal occurs in all directions, the degree dependent on local prevailing conditions during a small time increment and the availability of newly hatched larvae in a dispersive mode.

Summary

William E. Wallner and Michael L. McManus

The gypsy moth in North America operates in a bimodal fashion, having stable modes (outbreak and innocuous) and transient modes (release and decline) that are periodic in occurrence. This is in contrast to gypsy moth populations in Europe, which have been described as being cyclic and tending to increase from innocuous to outbreak levels every 7 to 9 years. In North American populations, no single factor is completely responsible for limiting gypsy moth at any one density. Some factors at each density are more important than others. While they may vary among geographic locations and from year to year, their relative importance can be generally characterized.

Predatory mortality is promulgated by factors, of which many may be random in occurrence. Predators, both vertebrate and invertebrate, exert the most general regulating effect in maintaining sparse populations. Predators are opportunists; however, their effects may differ widely among areas and may vary considerably from year to year. Since it appears that gypsy moth is not a preferred host by most predators, other conditions will influence predation. Factors such as available primary foods, weather, habitat, etc., will modify predator effects. The extent to which this affects the release of gypsy moth populations needs to be further explored with particular reference to shifts occurring in gypsy moth predator habitats over time.

The release phase, during which gypsy moth populations increase dramatically, have certain characteristics: Exceptionally large egg masses, a preponderance of female pupae, and a healthy, vigorous state. Given favorable weather conditions and adequate host numbers and quality, the population exceeds the limits of predatory influence and normally proceeds unimpeded for a 2- to 3-year period. However, qualitative changes in the host based upon the propensity of defoliation influences the population. Pupal weights are reduced, the developmental period is altered, mortality increases, and in general, the population is under physiological stress. Premature population reduction could be promoted by complete defoliation too early in larval development, causing the larvae to starve. However, in most cases larvae feeding upon hosts that are in the process of being defoliated suffer from a higher than normal incidence of physiological (nonpathological) mortality. The nutritional requirements of gypsy moth are poorly understood, yet they are believed to be an important factor in its population dynamics. It is known that the quality of the foliage of individual trees within the same location varies and that certain trees will experience above average defoliation. Thus, the apparent differential in nutritional value of the foliage is believed to influence survival and fecundity as well as to affect the vulnerability of gypsy moth to physiological dysfunctions. Further research is essential in determining the relationship between viral latency and the role nutritional imbalance plays in disease expression.

Drought has been reported to cause a shift in populations from the innocuous mode to the release phase. This is considered particularly critical in newly infested regions where populations of natural enemies are low. Gypsy moth populations can erupt from densities and cause no noticeable defoliation to complete defoliation in a single generation. Possibly, trees growing on xeric sites closely approximate continual droughty conditions and would serve as foci for incipient populations. Regional outbreaks might progress from such foci given appropriate weather stimulus. Defoliation maps show how outbreaks develop and progress from spot infestations to regional phenomena and indicate the probable role of larval dispersal. For example, in Connecticut during 1969-74, the outbreak did not arise from a single focus or hot spot but from a number of foci that expanded and then coalesced within a 1- to 2-year period. Similar defoliation and dispersal patterns have been observed in Massachusetts and Pennsylvania.

Mortality due to dispersal is probably high but has not been quantified. In natural situations, many larvae will be deposited in places where they cannot survive (water or pastures), while others will end up on either unfavorable hosts (for example, ash or tulip poplar) or favorable hosts that are unacceptable. Each dispersal episode uses energy reserves, and one can therefore assume that many unfed larvae will eventually encounter acceptable hosts, when they (larvae) are in a poor physiological state; this also may be detrimental to their survival. Certainly the geographical area of contiguous forest and the proportion of preferred hosts present will greatly influence the survival of dispersing larvae. All of the conditions discussed above must be considered when evaluating the role of dispersal in the dynamics of the gypsy moth.

Decline of the populations from the outbreak mode may result from adverse weather—abundant precipitation during the larval stage or absence of snow cover coupled with low winter temperatures. Spring weather conditions after eggs hatch and before larvae commence feeding can also severely affect larval survival. Periods of cool, wet weather force larvae to remain on masses, preclude migration to the foliage to feed or disperse, and result in significant mortality. Newly hatched larvae have remained on egg masses for up to 2 weeks under these conditions. Host insect synchrony further complicates survival. In years when eggs hatch prior to bud burst, larvae have inadequate food resources. Spring frosts, which kill new foliage similarly reduce the food source available. Perhaps the most important meteorological factor is the influence of spring thaws upon eggs that have completed diapause. Extended periods of warming (1 week or more) followed by freezing are believed to significantly affect egg survival. Additional research on this factor is needed because it is an important consideration in predicting establishment and expansion of populations in coastal or other areas modified by large bodies of water and regions in the southern part of the gypsy moth range. Additional research is needed on genetic and adaptive variation leading to high egg survival and the effect of temperature fluctuations on diapausing eggs. The relationship of gypsy moth to its host and those exogenous factors influencing the value of the host as a food source also require further definition.

Parasites contribute to overall mortality but by themselves are not considered the most significant factor. Some, such as *Parasetigena silvestris*, *Compsilura concinnata*, *Anastatus disparis*, and *Blepharipa pratensis* have the potential to be important in latent populations. Others, such as *Ooencyrtus kuvanae*, *Apanteles melanoscelus*, *B. pratensis*, and *Brachymeria intermedia* are associated with building or dense populations. This suggests that they are unable to maintain latent populations but capitalize on those in an expanding or outbreak mode. Experience with expanding gypsy moth populations in North America has demonstrated that parasites apparently do not initially play a vital role. However, certain species such as *Parasetigena silvestris* are belived to contribute to stabilizing populations during the first and second postoutbreak years. Parasites can serve as important vectors of disease; this relationship requires further definition not only in dense but also in sparse gypsy moth populations.

Nucleopolyhedrosis virus (NPV) is not considered to be a significant regulating mechanism in sparse populations. However, NPV is the single most important agent that causes declines of dense ones. Factors predisposing larvae to NPV infection and the ability to diagnose NPV in the larval population prior to an epizootic are critical to a more thorough understanding of gypsy moth population dynamics. Additionally, further research is needed to define the relationship between physiological disease and NPV latency and expression. Specifically, environment and host nutrition as predisposing factors require elucidation. Finally, the relationship of NPV latency to the genetic configuration of gypsy moth and variability in NPV susceptibility between different North American populations require definition.

Research completed within the gypsy moth program has reaffirmed the complexity of gypsy moth population dynamics. It is obvious that the life system is sufficiently complex so that few unequivocal assertions can be made without qualification. However, sufficient intrinsic knowledge is now extant so as to provide the fundamentals to intensify efforts on these critically identified research themes.

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Effects of Defoliation on Trees and Stands

<div style="text-align: right;">**5**</div>

Oak Decline and Mortality

David R. Houston

When enough leaves are removed too often from oak trees, the trees will die back, decline, and often die. Many casual observations and many excellent studies support this statement (Baker 1941, Beal 1926, Bess et al. 1947, Burgess 1922, Kegg 1971, Nichols 1968, Staley 1965, Stephens 1971). Therefore, is another treatise on defoliation-initiated oak decline and mortality necessary? The answer to that question must be judged by how well we have fulfilled our objectives to advance understanding of the underlying reasons why defoliated trees die and to provide practical means of assessing effects of defoliation and for predicting where and when trees and forests may be affected.

General Historical Background

The tree disease condition known as oak decline and mortality has occurred in many different places and at many different times in the oak forests of the Eastern United States and western Europe. Many accounts of this disease ascribe as causal factors spring frosts, drought, and insect defoliation, sometimes operating singly but more often interrelated directly or indirectly (Houston 1971). The relative importance of one or more of these climatic factors, along with outbreaks of host-specific insects, is a major reason why one oak species or one oak group is often more affected than another in a given episode. For example, over 910,000 m of white oak died in the valleys and hollows of North Carolina in 1926 as a consequence of a severe frost in May 1925. The frost affected the young, expanding white oak leaves more than the fully developed leaves of the red oak group (Beal 1926). A serious drought in July and August 1925 severely damaged or killed many of the black, red, and scarlet oaks on the ridges and upper slopes (Hursh and Haasis 1931). And, to make matters even worse, severe late frost in April 1927—at a time when foliage of the red oak group was probably susceptible—further affected these trees. Hursh and Haasis (1931) concluded that these two adverse environmental factors reduced the vigor of trees and predisposed them

to attack by the shoestring fungus, *Armillaria mellea* (Vahl ex. Fr.) Kummer (*Armillariella mellea* (Vahl ex. Fr.) Karst), the twolined chestnut borer, *Agrilus bilineatus*, and long-horned beetles.

A relationship between drought and frost was reported in Russia (Boylcevcev 1967). Injury to sapwood by frosts (ring rot) was greatest after 2 years of drought and especially when severe frosts were preceded by drought in the latter half of the growing season. Oak decline and mortality has thus been associated with the occurrence of several adverse environmental factors in combination with organisms of secondary action.

Widespread declines of oak have also resulted when frost, drought, or other adverse factors, singly or together, have occurred in association with severe insect defoliation. In Switzerland, oaks in the Save Valley declined when spring floods and silting were followed by gypsy moth infestation and attack by the fungus *Oidium quercinum* Thum (Barbey 1937). In Russia, a decline of oaks was correlated with droughts of 1921, 1948, and 1956, followed by defoliation in 1927 and by browntailed moths (*Nygmia phaeorrhoea* (Donov.)), gypsy moths, and leaf rollers (Minikevich 1962) in 1928, 1953, and 1959. The weakened trees were predisposed to vascular pathogenic fungi.

In the United States, oak frequently has exhibited decline on a massive scale. In general these occurrences have involved adverse environmental factors, particularly drought, defoliation by insects or hail, and finally attacks of weakened trees by root fungi and bark borers. Decline of oak over extensive areas of the Southeast occurred in the early 1960's. This area had suffered from a series of dry years in the middle 1950's and probably from attacks of the elm spanworm and cankerworms.

A decline of red and scarlet oaks occurred from Pennsylvania to North Carolina. An intensive study of this problem by Staley (1965) revealed that decline resulted from leaf roller defoliation possibly aggravated by drought and late spring frost and by attacks of *Agrilus* and *Armillaria*. Essentially the same conclusions were reached by Nichols (1968).

<div style="text-align: right;">217</div>

Periodic declines of white oak in the Northeast have also followed similar combinations of adverse factors.

Symptoms of oak decline initiated by a number of causes, including defoliators, are similar and include early-season chlorotic foliage; crown thinning resulting from progressive dieback, initially of upper crown buds and twigs and eventually of large branches; and the development of sprouts along the bole and at branch nodes. Associated with these external symptoms are a reduced or depleted reserve of starch and a reduction in radial and terminal increment. These symptoms are common to many diebacks-declines (Houston 1967, 1973, 1974). Sometimes badly affected oaks die over winter and do not leaf out the next spring, but often death occurs in the midsummer to late summer. When this happens, the leaves wilt, turn brown, and hang on the tree, often well into next year. Galleries of *Agrilus bilineatus* and mycelial fans and rhizomorphs of *Armillaria mellea* are common beneath the bark of dead and dying trees. Symptoms of oak decline and mortality following leaf roller defoliation have been described in detail by Staley (1965), and recently, both the symptoms and organisms of secondary action of oak decline following gypsy moth defoliation have been described and illustrated (Wargo 1978*d*).

Historical Background of Oak Decline and Mortality Associated With Gypsy Moth Defoliation in the United States

Decline and death of oaks has been associated with the gypsy moth ever since the first outbreaks of this insect occurred in eastern New England. The early reports of tree losses in this country are dramatic accounts of the destruction of a susceptible forest by the first attack of an introduced pest (Baker 1941, Burgess 1922).

Analyses of data collected in eastern New England from 1911 to 1931 (Melrose Highlands Study) have revealed mortality patterns that are of interest today as an attempt is made to interpret the recent first encounters of the insect with the forests of New Jersey and Pennsylvania (Campbell and Valentine 1972 and

Campbell and Sloan 1977). Briefly, it was quite clear that the fate of a defoliated tree was determined primarily by its condition when it was defoliated and by the number of years it was defoliated severely. Thus, 35 percent of the oaks classified in poor condition died after one severe defoliation compared to but 7 percent of those in good condition. After 2 successive years of defoliation, the results were even more dramatic: 22 percent of the oaks rated as good and 55 percent of those classified as poor died within 5 years. Overall, the forests of the Melrose Highlands Study that contained mostly oaks lost about half their trees from 1911 to 1921.

Defoliation and the responses to it were markedly less during the second decade of the Melrose Highlands Study (Baker 1941, Campbell and Sloan 1977). Indeed, a sustained outbreak of the magnitude that occurred from 1911 to 1921 has not been recorded since in New England. Outbreaks have occurred since 1931 in each of the New England States, but their frequency, duration, and consequent tree mortality appear less than the earlier ones (for example, Stephens and Hill 1971).

On the other hand, reports from New Jersey (Kegg 1971, 1973) and Pennsylvania (Nichols 1961) reveal that high tree mortality has followed recent outbreaks there. Studies conducted during the gypsy moth program confirmed that in some forests mortality has indeed been high following the recent outbreaks; these studies also reveal that in some forests mortality has been quite low. Nearly every account of the decline and death of oaks following gypsy moth defoliation in this country has associated dying and dead trees with the presence of *Agrilus bilineatus* and/or *Armillaria mellea* (for example, Baker 1941, Dunbar and Stephens 1975, Nichols 1961). The role of these organisms of secondary action in oak decline and mortality and the relationship of defoliation stress to this attack are covered in detail in following sections of this chapter. Similarly, an implication of many studies is that defoliation is more damaging when it occurs in concert with drought (Bess et al. 1947, Campbell and Sloan 1977, Stephens and Hill 1971). The effects of drought and defoliation on trees are also discussed in later sections of this chapter.

In general, then, oak decline and mortality occurs when oaks, weakened by severe defoliation for 1 or more years, are attacked and killed by the girdling actions of *Agrilus bilineatus* and/or *Armillaria mellea*. The following sections of this chapter present the results of studies to determine the nature of the biochemical effects of defoliation on trees; to measure these effects and use some of them as indicators of stress and predictors of tree vigor; to clarify the relationship of these effects to attack by organisms that kill trees; and to determine ways to classify stands with respect to their susceptibility to the gypsy moth.

Individual Tree Relationships

Effects of Defoliation on Oak Chemistry

Johnson Parker

Introduction

What happens to an individual tree when it is defoliated depends to a large extent on how much foliage is removed (severity); on the number of successive years of defoliation (frequency); on when in the growing season the tree is defoliated (timing); on the presence and number of secondary organisms (pathogens and insects); and on the physiological condition of the tree when it is defoliated (health and vigor). These are the crucial factors, although there are probably many more involved, such as weather and site conditions. It is the interaction of these factors that determines the ultimate effects of defoliation, but it is defoliation that begins the process (Wargo 1978*b*).

Carbohydrates

Carbohydrates, in terms of volume or weight, are the most important group of compounds in forest trees. Of the three major types of carbohydrates— starch, sugars, cellulose—starch comprises most of the stored, metabolically available carbohydrate in the normal healthy tree. Free sugars make up only a small portion of the available carbohydrates. Cellulose and other cell wall components, although constituting a large proportion of the tree, are largely inert. Although insoluble under most conditions

outside the cell, starch can be converted by enzymes into components available to the respiratory system, thus assuring continuation of the living processes when the food supply is low. In the research on changes within a tree following defoliation, it was therefore inevitable that starch would become one of the main objects of study.

When this study was begun in 1966, almost nothing was known about the effects of defoliation on organic metabolites. Sugar maple (*Acer saccharum* Marsh.) was used in the initial studies of the role of defoliation stress in dieback and decline diseases, because of its economic and ecological importance in the Northeast and because of its known susceptibility to defoliation-initiated dieback-declines (Houston 1967). Total food reserves in shoots and roots were examined first.

Defoliation of sapling-sized trees was carried out in June, July, or August 1966 by stripping all leaves from the trees by hand. Other trees were left undefoliated as controls. Some trees were defoliated twice in the same season and some again in 1967 and 1968. Harvesting of root collars and whole roots was carried out at various times from July to November 1966 and 1967, and from August to November 1968.

Before defoliation, larger quantities of food reserves were found in roots than in above-ground parts. Defoliation caused a decline in root food reserves, most of which were in the root wood (and some in the root bark) (fig. 5-1), and most of the decline in the wood could be accounted for by loss of starch.

In general, the trees that were defoliated in mid-June or mid-July had sharp drops in food reserves, as compared to the controls, whether harvested in midsummer, late summer, or early autumn. Defoliation in late August, however, affected food reserves very little.

Successive defoliations for 3 years resulted in a downward trend in the food reserves that could be extracted from roots. In addition, the trees grew progressively less in diameter as defoliation severity increased. This indicated that the trees could compensate, in a sense, for a lowered photosynthesis by laying down less carbohydrate as cell wall material (Parker and Houston 1971).

Other parts of the trees did not show much change in food reserves with defoliation. Root-collar extract levels were practically the same for all treatments (fig. 5–1) (Parker and Houston 1971).

The apparent importance of root-wood tissue as a storehouse for starch led to examination of the various woody tissues of the root with light and electron microscopes. Distinct areas of specialized living cells were discovered in the root wood of sugar maple. These are evidently capable of losing and regaining starch several times over a period of several years (Parker 1975*a*).

These studies also revealed that a marked decline in starch was accompanied by increases in fructose and glucose (Parker and Houston 1968, 1971). Investigations by others showed a similar decline in starch and an increase in certain sugars like glucose in plants subjected to drought (reviewed by Levitt 1956). The question then arose as to whether other stresses, such as drought, would have an effect on trees similar to the effect of defoliation.

An experiment was designed to analyze roots of 2-year-old *Quercus velutina* Lamarck seedlings for changes in starch and sugars following defoliation, drought, or the combination of both. Results clearly showed that either drought or defoliation tended to increase reducing-sugar levels, and the two stresses together generally accentuated this effect (fig. 5–2) (Parker and Patton 1975). These findings seem to verify field observations that drought and defoliation can combine in nature to bring about more damaging effects than either one alone (Baker 1941, Houston 1967).

Although the causes of the metabolic changes in carbohydrates initiated by defoliation are not yet clear, a pattern is discernible. When a tree is defoliated, carbohydrate production and presumably its transport to the roots are curtailed. Starch begins to decline in the roots about 2 weeks after defoliation (Wargo 1972). Starch may be made up again by subsequent photosynthesis after refoliation. The twigs themselves, although containing chlorophyll, contribute only a small amount of photosynthate to the total (Parker 1975*b*).
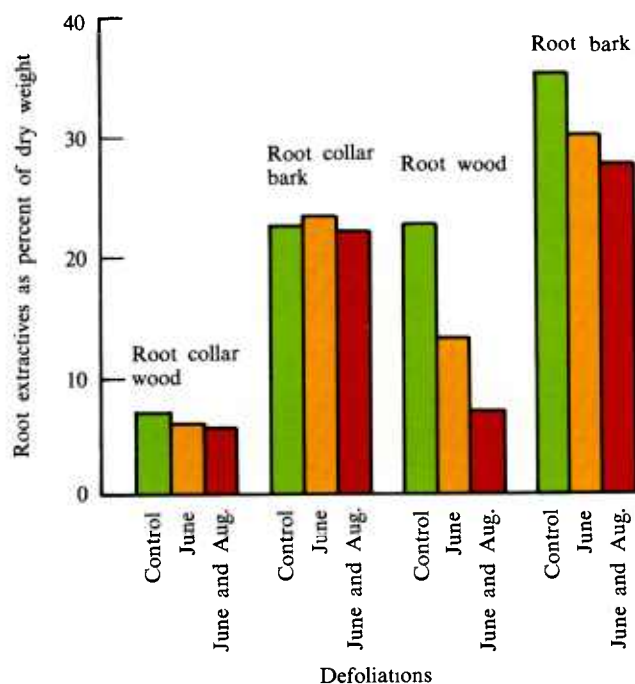
Figure 5–1.—*Extracts as a percent of dry weight for tissues of control and defoliated trees. All trees were harvested in November of the same year. Each bar represents the average of five trees. (Parker and Houston 1971.)*
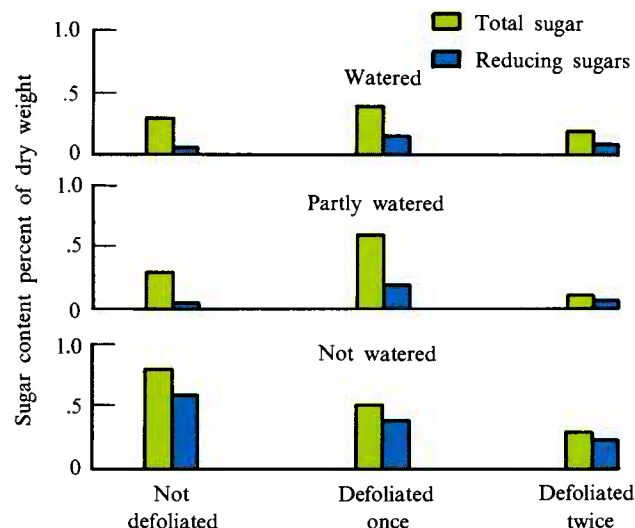
Figure 5–2.—*Influence of drought and defoliation on sugar content of black-oak roots. Each bar represents sugar content as a percent of dry weight, one sample per seedling. N =10. (Parker and Patton 1975.)*

The cause of the root starch decline and the subsequent rise in certain sugars may be the production of a hormone such as a gibberellin. On the other hand, the starch/sugar conversion mechanism may be triggered simply by the decline in glucose as it is used up in respiration and growth. Then, as the enzymes that hydrolyze starch are activated, starch is gradually converted to glucose, and the level of this sugar increases in spite of continued respiration.

Fructose also increases as a result of the enzymatic conversion of some of the glucose to fructose. It is probable that there is then an excess of glucose (and therefore of fructose) produced from starch as an overshoot effect often seen in enzymatic reactions. These simple reducing sugars can be used by the living cells in respiration, cell wall formation, and conversion to sucrose or other oligosaccharides, in which form they move up through the tree, either in the phloem, xylem, or both (Parker 1974a).

A marked rise in glucose in the roots may help to explain why certain fungi can readily invade and kill the roots of defoliated trees. Glucose is known to be an especially suitable energy source for the root-rot fungus *Armillaria mellea* (see Phenols, next section).

Sucrose, which occurs in a larger quantity than other sugars, did not change after defoliation as the reducing sugars did. Instead, usually it declined progressively under increasingly severe stress conditions. It behaved, then, something like a storage carbohydrate. The effect of the combination of drought and defoliation on sucrose was usually greater than the effect of either one alone (Parker and Patton 1975). Raffinose, a related but less abundant trisaccharide, behaved similarly.

It has been known for a long time that starch may temporarily decline in spring when leaves emerge, then build up again shortly afterward (Levitt 1956). The question then arises, is the decline in sucrose and starch that occurs naturally in late spring and early summer, as a result of seasonal changes related to bud burst, similar to that following stress? A study of seasonal sugar changes in *Acer saccharum* roots (Wargo 1971) showed that there is a decline in sucrose, raffinose, and stachyose in spring, whereas maltose declines earlier (fig. 5-3). There is also a tendency for

starch to decline at this time. Then, in the next few months, sucrose and starch rise again to former levels. Sucrose reaches an annual peak in summer, and stachyose and raffinose in November. These changes, however, did not appreciably affect the results of the defoliation experiments.

Other sugars occur in these oaks and maples as well as in other common forest tree species of the North-eastern States (Parker 1974b). In *Q. velutina,* for example, xylose, mannose, and arabinose were present (Parker 1974b, 1978).

A number of so-called sugar alcohols or polyols were also found in oak leaves, mainly by means of high-pressure liquid chromatography. Although known previously to occur in many vascular plants, most of them had not been observed in native oaks and maples prior to this study. Inositol, sorbitol, and mannitol were found in oak leaves (Parker 1978), but presence in other parts of the tree has not yet been determined.

Amino Acids

It has been known for at least a half century that nitrogenous compounds fluctuate in living cells of trees with the changing seasons (for example, Levitt 1956). Certain nitrogenous compounds like the proteins in forest trees of a north temperate climate increase in autumn and decline in spring (Parker 1958). At the same time, some free amino acids may decline while others rise.

The effects of defoliation on nitrogenous compounds in trees have remained unknown until this decade. Studies with *A. saccharum* tree roots following total defoliation revealed that some amino acids increased: Threonine, cysteine, tyrosine, histidine, and proline (Wargo 1972). Subsequent studies on roots of *Q. velutina* seedlings showed that either defoliation or drought could cause a rise in levels of certain amino acids (table 5-1) (Parker and Patton 1975). Most amino acids that increased with defoliation also increased, and often more so, with drought.

Nothing was known about the effect of drought on amino-acid levels in maples and oaks in the late
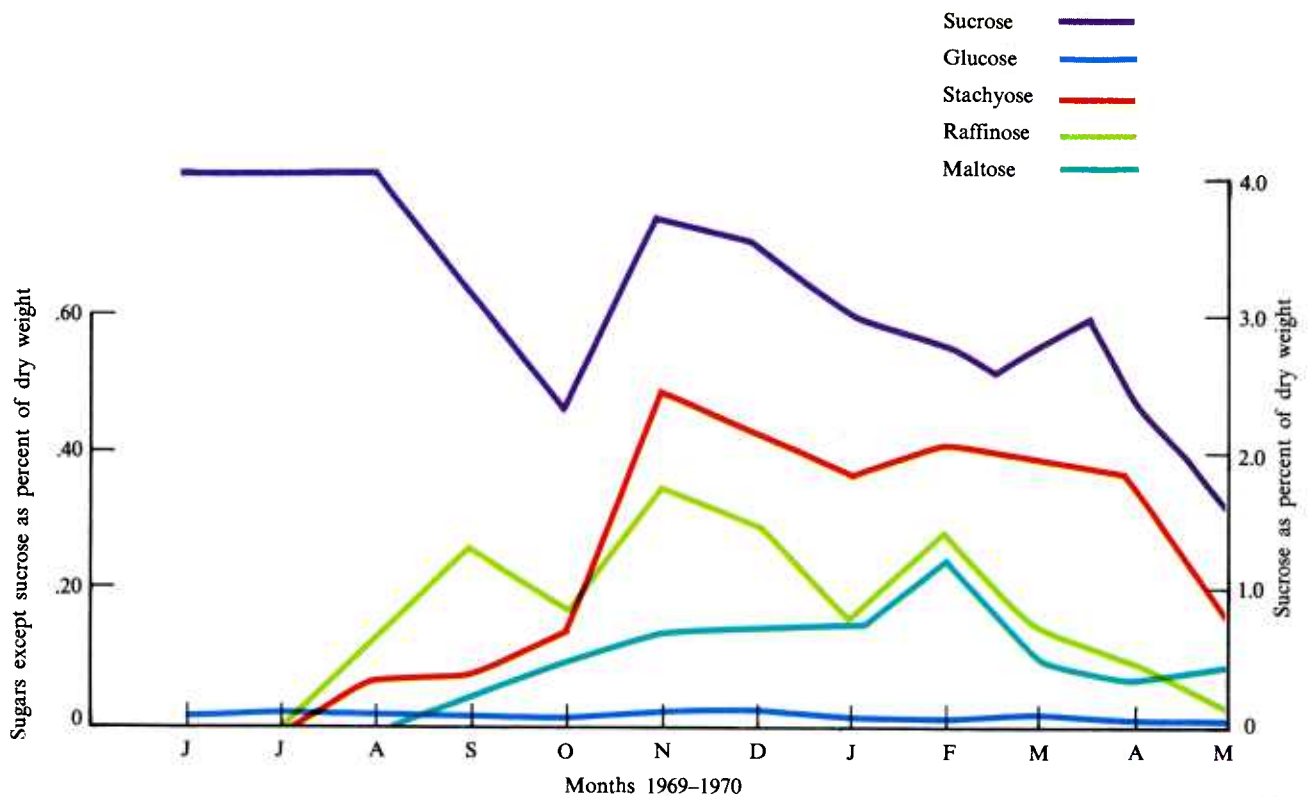
Figure 5-3.—*Seasonal trends of sugars in outer root wood of sugar maple. (Wargo 1971.)*

1960's. In the research, drought was found to have a marked effect in increasing the already high levels of asparagine (Parker and Patton 1975) (table 5-1). Proline, normally very low in concentration compared to other amino acids, increased greatly. Interestingly, a synergistic effect between drought and defoliation was again shown: Asparagine was twice as high in roots of twice-defoliated, droughted seedlings as in twice-defoliated, nondroughted ones (Parker and Patton 1975) (table 5-1).

As mentioned before, changes in amino acids after defoliation may contribute considerably to the susceptibility of oaks and maples to attack by *A. mellea,* since this fungus is greatly stimulated in its growth by asparagine as well as glucose. Wargo (1972) found that growth of *A. mellea* was greater in all media used when amended with extracts of the outer

root wood of defoliated *A. saccharum* trees, but not in all media amended with bark extracts of this species. The fungus always grew better on extracts of root wood from defoliated trees than on those from nondefoliated ones. Obviously, something, or lack of something, in the root wood of defoliated trees encourages growth of *A. mellea.* This difference might be accounted for by the combined effects of increased glucose and certain amino acids like asparagine and a change in phenolics.

Phenols

Phenols are an important component of forest trees because they seem to aid in the defense of the tree against insects, fungi, and bacteria, serve as a source of food reserves, and are the basis for the wide array of

Table 5-1.—*Amino acids in roots of black oak seedlings.*[1]

	Amino acid	Not defoliated	Defoliated once	Defoliated twice
		Milligrams per gram of dry weight (N = 10)		
	Alanine	0.09	0.15	0.20
	Asparagine	.40	.75	.75
	Glutamic acid	.50	.51	.46
Watered	Leucine		.01	
	Phenylalanine		.04	.04
	Proline	.01	.07	.13
	Tyrosine	.01	.07	.09
	Alanine	0.23	0.40	0.16
	Asparagine	.70	.80	.75
	Glutamic acid	.64	.63	.41
Partly	Leucine		.01	.31
Watered	Phenylalanine		.02	.07
	Proline	.01	.06	.04
	Tyrosine		.10	.07
	Alanine	0.48	0.12	0.02
	Asparagine	2.60	1.50	1.40
	Glutamic acid	.37	.13	.02
Not	Leucine	.01	.01	
Watered	Phenylalanine		.02	
	Proline	.27	.02	.01
	Tyrosine	.05	.05	.01

[1]Only those showing statistical significance among watering treatments are shown. Only proline showed significant differences among defoliations.

colors found in the plant kingdom. A flavonoid such as quercitrin, which imparts the yellow color to the bark of *Q. velutina*, can average up to about 4 percent of the fresh weight of *Q. velutina* twig bark (Parker 1974*b*), whereas a free sugar in similar tissues can account for about 0.1 to 0.5 percent of the fresh weight. In roots, however, phenolics may occur at somewhat lower levels.

Phenols and their various relatives, including tannins, occur mainly in vacuoles of most living tree cells and also in cell walls of wood, bark, and leaves. Most all are chemically bound to sugars like glucose, rhamnose, and galactose. In bark, the best known phenolics and the most difficult to characterize are the tannins.

A survey was conducted of six different species of common forest trees—*Q. velutina* Lamarck, *Q. alba* L., *Q. rubra* L., *Fraxinus americana* L., *A. saccharum* Marsh., and *Carya glabra* (Mill.) Sweet.—to determine the identity of the principal phenolics in bark and wood of stems, roots, and leaves and to find out how much the phenolics differed among the various species. The effects of defoliation on certain species, especially the oaks, could then be studied.

Two kinds of tannin were found in leaves and bark of all species except *F. americana*: Condensed tannin and hydrolyzable tannin (Parker 1974*b*). The other tree species could be identified by the kinds and quantities of phenolics they possessed. Numerous flavones (fig. 5-4) and flavanols were found in each species. The occurrence of different radicals or substituents on the phenyl rings characterized them as different compounds and separable by the various chromatographic methods.

In *Q. velutina* there were about 30 phenolics of the flavonoid or catechin type and about 22 blue or purple-fluorescing compounds (such as scopoletin and chlorogenic acid) observable under ultraviolet illumination as spots on chromatograms (Parker 1977). Possible pathways in the formation of these compounds (based on various sources, including Birch 1962) are suggested in figure 5-4.

The effect of defoliation on the phenolics was studied in both oak seedlings and pole-size trees. In *Q. velutina* seedlings (Parker and Patton 1975), catechin and sometimes quercitrin (quercetin 3-O-rhamnoside) declined with defoliation (fig. 5-5). The effects of drought were less certain: Changes with different treatments were not statistically significant.

In another study with *Q. rubra*, in which seedlings were kept at different heights above a water table, drought and defoliation each caused a decline in chlorogenic acid and catechin, the only phenolics quantified (Parker 1978). Declines in these compounds have been related in a number of plants like potato and tobacco to the invasion of certain fungi (Kuć 1963, Hare 1966). Decline of phenolics in roots of *Q. rubra* following defoliation suggests the possibility that it may be related to an increase in
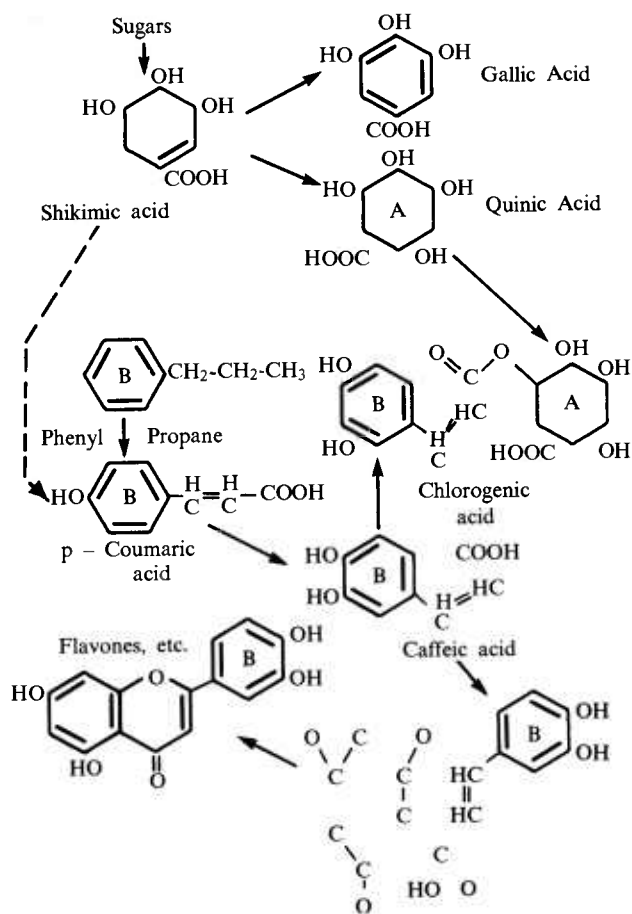
Figure 5-4.—*Some possible pathways to show relationships among sugars, shikimic acid, and some common phenolics found in oaks and maples. Numerous steps have been omitted and some steps simply suggested.*

Figure 5-5.—*Percent of catechin or quercitrin in roots of seedlings, based on dry weight. N = 8. Only catechin was significantly different among defoliations. (Parker and Patton 1975.)*

susceptibility to root pathogens. Although phenolics in some plants are known to increase following infection (Kuć 1963), the presence of preformed fungal inhibitors is still recognized as important to disease resistance. Current studies indicate that it is likely that these inhibitors in oaks include tannins and compounds chemically bound to the tannins, like gallic acid. It has not been possible as yet to show that tannins decline in defoliated oaks. In one study with pole-size *Q. velutina* trees, there was a decline in chlorogenic acid in the branch bark, following two defoliations, but not in the roots, where *A. mellea* would attack (Parker 1976).

In a series of experiments, an attempt was made to answer the question of whether the more prominent phenolics found in oaks would, in fact, inhibit growth of *A. mellea.* Although some such experiments had been tried many years ago (Cook and Taubenhaus 1911), various phenolic concentrations and different strains of *A. mellea* had never been employed. Most of the experiments were carried out with a fungus isolated from sugar maple in New York State. It was

found that gallic acid,, a component of oak tannin, and tannin similar to hydrolyzable tannin in oak bark inhibited growth of *A. mellea,* but only in concentrations near 2 percent. Catechin, quercetin, quercitrin, and chlorogenic acid, however, stimulated growth, especially at concentrations near the 2 percent level. Each of five other isolates of *A. mellea* of various origins (isolated from different species of trees) reacted differently to the phenolics. Only one of these isolates was inhibited by gallic acid and tannin and not nearly as strongly as the sugar maple isolate (Wargo and Parker 1977).

A conclusion may be reached that increases in glucose and certain amino acids following defoliation could be at least one of the causes of success of attack by *A. mellea.* However, a decline in several phenolics following defoliation—phenolics that have been shown in other studies to be important in preventing or delaying invasion—makes it seem possible that phenolic defense mechanisms may also play a role. Other compounds may also be important. There are enzymes produced by the host tree that are known to be able to lyse, or dissolve the cell walls, of invading *A. mellea* hyphae (Wargo 1975). In fact, some evidence exists that defoliation decreases the host's enzymatic activity, although statistical evidence for this was lacking (Wargo 1976). Hormones, too, could play some role, but the weight of evidence from other plants suggests that sugars, amino acids, phenolics, and enzymes are most often closely involved in determining the susceptibility of plants to parasitic fungi.

Defoliation and Tree Growth
Philip M. Wargo

Effects on Leaf Production

The effects of defoliation on growth and mortality of trees after insect defoliation are well documented (Staley 1965, Nichols 1968, Kulman 1971, Stephens 1971, Campbell and Valentine 1972, Kegg 1973, Campbell and Sloan 1977). However, surprisingly little information exists on the effects of defoliation on leaves, the life-sustaining system of the tree.

Symptoms of the effects of defoliation are often characterized by leaf appearance—for example, tufted and clumped chlorotic foliage and leaves smaller than normal (Houston and Kuntz 1964). These symptoms are associated with advanced dieback and indicate considerable response to defoliation. However, the leaf system is impaired long before these advanced symptoms occur.

When a deciduous tree is heavily defoliated in the early growing to midgrowing season it responds by producing a new flush of leaves. Thus in a defoliation/refoliation system there is a primary or original and a secondary or refoliated leaf system. Defoliation can, unfortunately, affect both systems.

Primary Leaves

Defoliation has a direct effect on the primary leaves. The area of each leaf is reduced according to the number of insects that are feeding; in many instances it is reduced to zero. Defoliation can also have an indirect effect on the primary leaves. Heichel and Turner (1976), in an intensive study on red oak and red maple, observed that spring budbreak in the first year after defoliation occurred earlier on defoliated red oak trees by as much as 7 days. In areas where frost is a problem, an earlier than normal bud break could expose the new leaves to frost damage in addition to another defoliation later in the growing season. Obviously, the double defoliation (first by frost and second by the insect) could have drastic effects on tree growth. Damage by frost alone can be tremendous (Beal 1926).

The growth of individual leaves is also affected in the years after defoliation. Heichel and Turner (1976) found that leaf area was reduced by defoliation and the percent reduction was greater as the percent of defoliation increased from 50 to 100 percent. The initial defoliation exerted the greatest effect on leaf area, and subsequent defoliations failed to cause successive reductions in leaf area. In a similar study,

significant reductions were also observed on the leaf area of the primary leaves.

Three species of forest trees were used in a study conducted from 1971 to 1975; black oak (*Quercus velutina* Lamarck), white oak (*Q. alba* L.), and sugar maple (*Acer saccharum* Marsh). Sugar maple, a diffuse-porous species, was included as a contrast to the ring-porous oak trees and because a great deal of information about this species' response to defoliation was already known (Giese et al. 1964*a,b,* Houston and Kuntz 1964, Parker 1970, Parker and Houston 1971, Wargo 1972, Wargo et al. 1972).

Young trees that had seeded into a recently cut stand (oaks) and into an abandoned field (maple) were used. These trees were chosen because they could be easily defoliated and intensively measured. Fifty trees of each species, from 3 to 6 cm in diameter at 1.4 m and 4 to 6 m tall, were randomly selected. Trees were 12 to 15 years old at the start of the study in 1971. Ten trees of each species were defoliated in mid-May, mid-June, mid-July, or mid-August, or not at all (controls).

Trees were defoliated manually and completely (100 percent of leaves removed) in 1971, 1972, and 1973. Only the primary leaves were removed.. The 100 percent level of defoliation was used because it results in refoliation. Defoliation severe enough to cause refoliation seems to have the greatest effect on tree physiology and growth (Wargo et al. 1972, Heichel and Turner 1976). Also, removal of all leaves eliminated any individual tree differences resulting from total leaf area remaining after partial defoliation.

At the time of defoliation, trees were bent over gently to form an arch and held in place with a rope attached to the main stem at midcrown and tied to a stake driven into the ground. While the tree was bent, the total number of leaf clusters and dead terminal buds were counted for each tree, and the length and width of the five largest leaves on each of four leaf clusters were measured to the nearest centimeter. Leaf blades were then removed by hand or with scissors, but the petioles were left intact, after which the tree was released and straightened. Primary leaves on trees

defoliated in mid-May were not measured during the 3 years of defoliation (1971–73) because they were less than 25 percent of their full size at the time of defoliation. Leaves were measured in a similar way on the undefoliated control trees in mid-June after complete leaf expansion. In 1974 and 1975, the years in which the trees were not defoliated, leaves on all trees were measured in mid-June.

New leaves (secondary) were produced on all trees defoliated in May, June, and July and on some August-defoliated trees. They were measured in early October in each year of defoliation (1971–73). Little or no refoliation occurred on most trees defoliated in August, and on most trees that did refoliate the leaves were small and too few to measure.

All length and width measurements were converted to area measurements using equations generated for each species to estimate leaf area (Wargo 1978*a*).

After 3 successive years of defoliation in May, June, July, or August, the average area of individual leaves on defoliated trees was significantly smaller than on undefoliated trees (fig. 5–6, *A, B,* and *C*). Compared each year to the undefoliated trees, there was a progressive decrease in leaf area after each defoliation in black oaks defoliated in June and July, white oaks defoliated in June, July, and August, and sugar maples defoliated in June. In both oak species, defoliation in June and July had the greatest effect on leaf area and in sugar maple June defoliation was most adverse. The pattern of leaf area change for trees defoliated in May was not determined because no leaf measurements were obtained until spring 1974. However, leaf area in 1975 was greater than in 1974, indicating that leaf area had also been significantly reduced on trees defoliated in May. Minimum leaf area occurred in 1974 after the third defoliation; in the absence of defoliation in 1974, leaf area stabilized or began to increase in 1975.

Leaf area was also affected in the undefoliated control trees (fig. 5–7, *A, B,* and *C*). Compared to their original leaf area in 1971, average leaf area of the control trees was smaller in 1972. The reduction was significant ($P=0.01$) in white oak and sugar maple but

not in black oak. There was a linear trend of recovery of leaf area in both species and it was significant for both species (fig. 5–7, *B* and *C*). The decrease in white oak and sugar maple may reflect some partial natural defoliation by the gypsy moth and elm spanworm that occurred in 1971.

The comparison of leaf areas in 1972, 1973, 1974, and 1975 to the original leaf area in 1971 within each defoliation treatment verified the trends indicated in the original comparison with undefoliated trees (fig. 5–7, *A, B,* and *C*). However, this second comparison emphasized that leaf area recovered after defoliation ceased—that is, the leaf area increased in 1975. This

comparison also showed that the effect of the first defoliation (1971) on leaf area was similar for all months of defoliation, especially in black oak and sugar maple. Leaf area reductions in 1972 on June, July, and August defoliated trees were 26, 28, and 31 percent for black oak; 19, 15, and 26 percent for white oak; and 29, 26, and 30 percent for sugar maple.

Secondary Leaves

Deciduous trees survive severe defoliations by producing a new flush of leaves. However, this secondary leaf system is inferior to the original set of
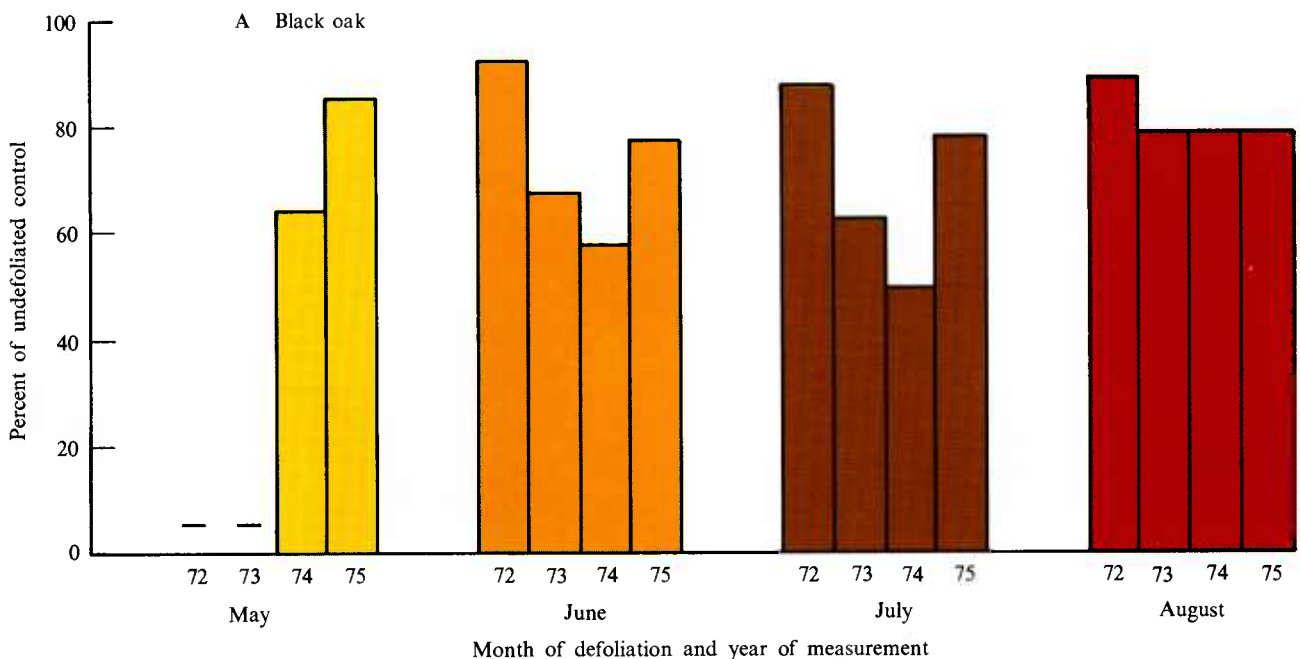
Figure 5–6.—*Change in average area of individual primary leaves after artificial defoliation (100 percent) in May, June, July, or August in 1971, 1972, and 1973, plotted as percent of the leaf area of the undefoliated control trees in each year. No measurements recorded for May defoliated trees in years of defoliation: A, Black oak—average leaf area* *of undefoliated trees was 94 cm² in 1972, 100 cm² in 1973, 95 cm² in 1974 and 105 cm² in 1975; B, white oak—average leaf area of undefoliated trees was 69 cm² in 1972, 73 cm² in 1973, 75 cm² in 1974 and 78 cm² in 1975; C, sugar maple—average leaf area of undefoliated trees was 58 cm² in 1972, 60 cm² in 1973, 65 cm² in 1974 and 80 cm² in 1975.*

Figure 5–6 B   White oak

Percent of undefoliated control

100 —
80 —
60 —
40 —
20 —
0 —

| 72 | 73 | 74 | 75 | 72 | 73 | 74 | 75 | 72 | 73 | 74 | 75 | 72 | 73 | 74 | 75 |

May   June   July   August

Month of defoliation and year of measurement

Figure 5–6 C   Sugar maple

Percent of undefoliated control

100 —
80 —
60 —
40 —
20 —
0 —

| 71 | 72 | 73 | 74 | 75 | 72 | 73 | 74 | 75 | 72 | 73 | 74 | 75 | 72 | 73 | 74 | 75 |

May   June[1]   July   Aug

Month of defoliation and year of measurement
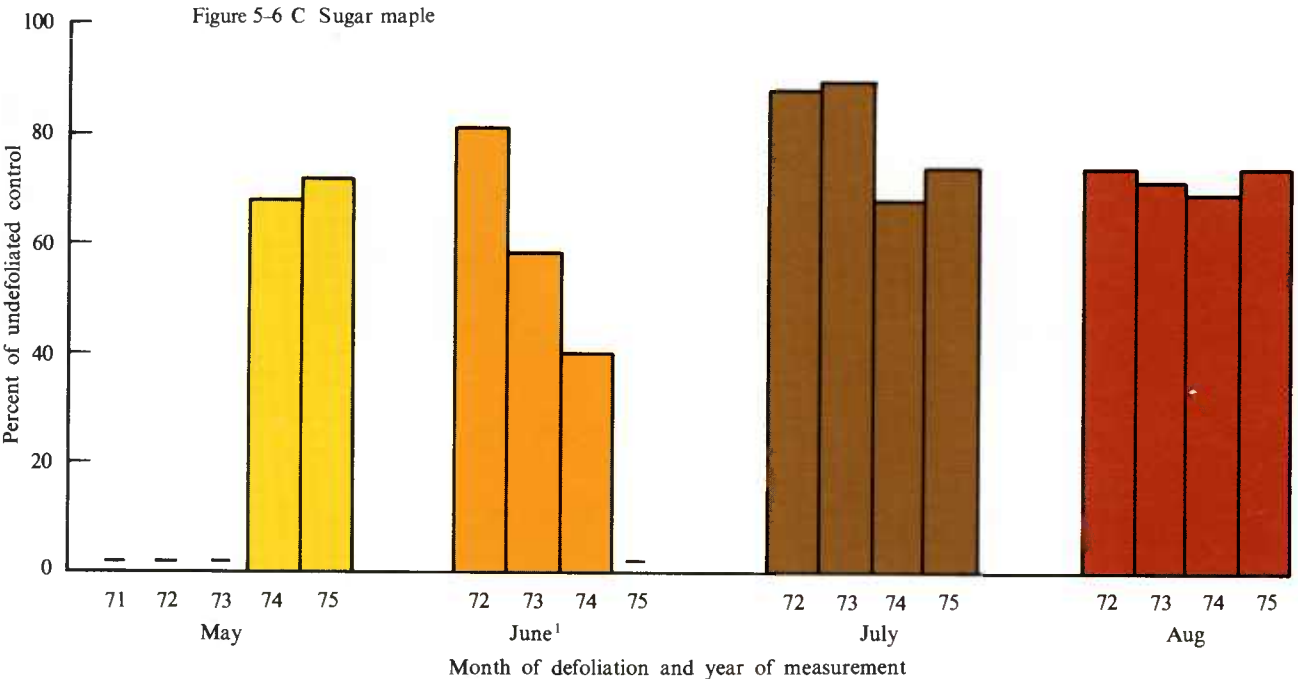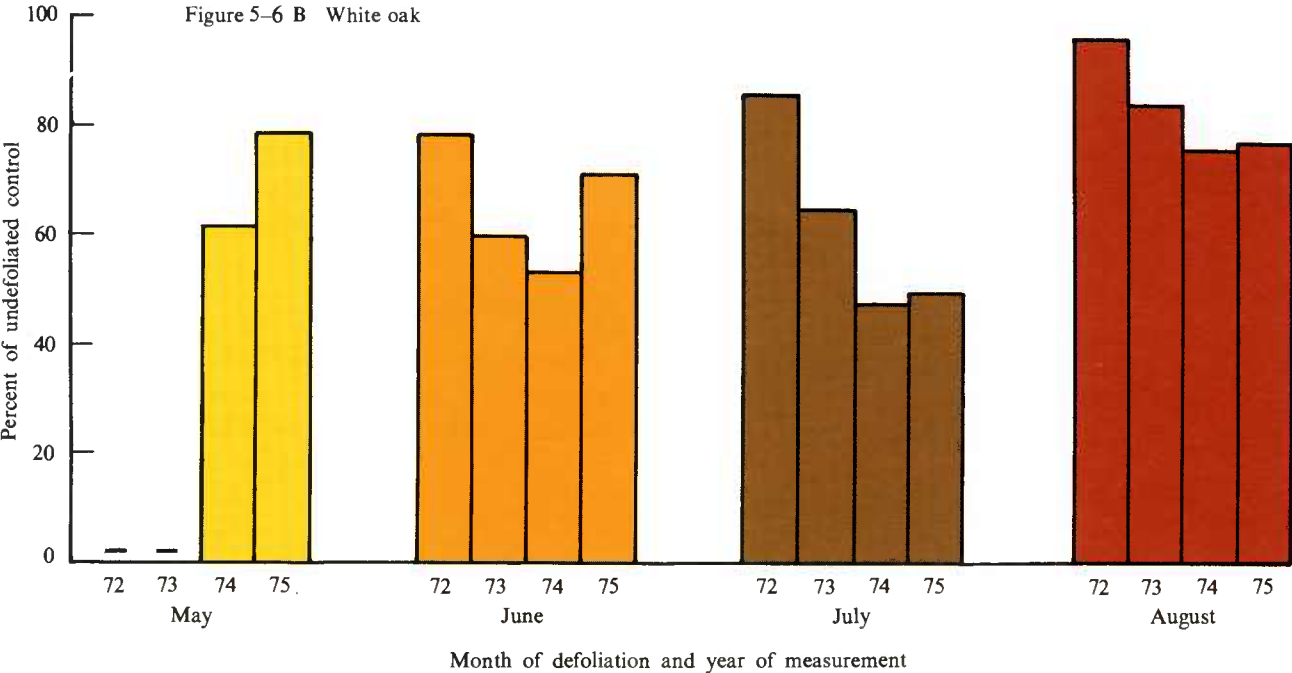
[1]All June defoliated trees dead by autumn 1974.

leaves. Both the number and size of the new leaves are significantly less than the primary leaves prior to defoliation (Heichel and Turner 1976, Giese et al. 1964b). Heichel and Turner (1976) observed a 30- to 60-percent reduction in leaf area and a 30- to 50-percent reduction in leaf numbers on red oaks after severe defoliation. This resulted in the trees having only 29 to 40 percent of the original total leaf area to produce food within a shortened growing season. Giese et al. (1964b) observed that refoliated leaves on sugar maples were 25 to 35 percent of normal size and

were only 35 percent of the normal complement of foliage.

Not only are the refoliated leaves smaller and fewer but repeated defoliation can cause additional reduction in leaf size. In the program study, the effects of repeated defoliation were observed in the secondary leaves produced in May, June, and July but not on the few trees defoliated in August that refoliated (fig. 5-8, A, B, and C). With the exception of trees defoliated in August, the secondary leaves of all three species were significantly smaller after the
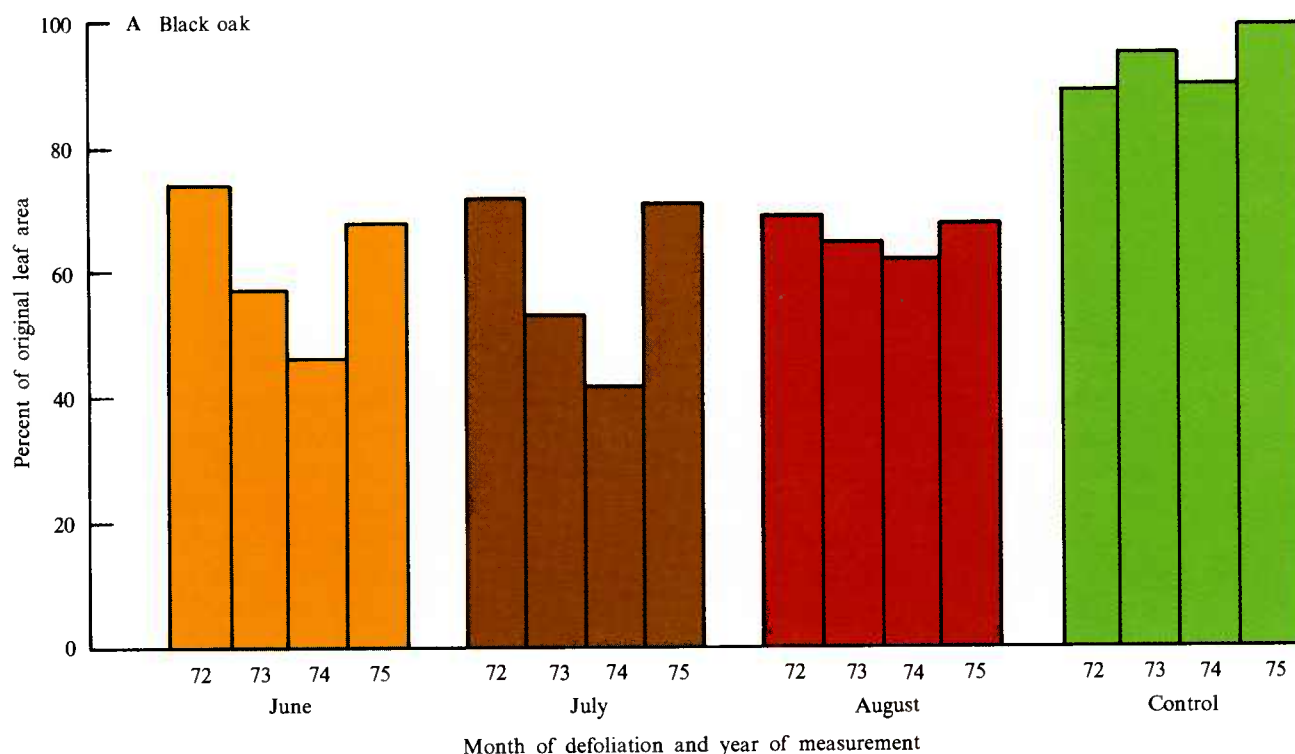
Figure 5-7.—*Change in average area of individual primary leaves after artificial defoliation in June, July, and August and control in 1971, 1972, and 1973, plotted as percent of the original average leaf area for each defoliation treatment in 1971. No 1971 data for May defoliation:* A, *Black oak—average leaf area in 1971 was 118 cm² for June defoliated trees, 114 cm² for July trees, 120 cm² for August trees and* 105 cm² *for control trees;* B, *white oak—average leaf area in 1971 was 65 cm² for June defoliated trees, 69 cm² for July trees, 90 cm² for August trees, and 91 cm² for control trees;* C, *sugar maple—average leaf area in 1971 was 66 cm² for June defoliated trees, 69 cm² for July trees, 61 cm² for August trees, and 66 cm² for control trees.*

Figure 5–7 B White oak

Month of defoliation and year of measurement
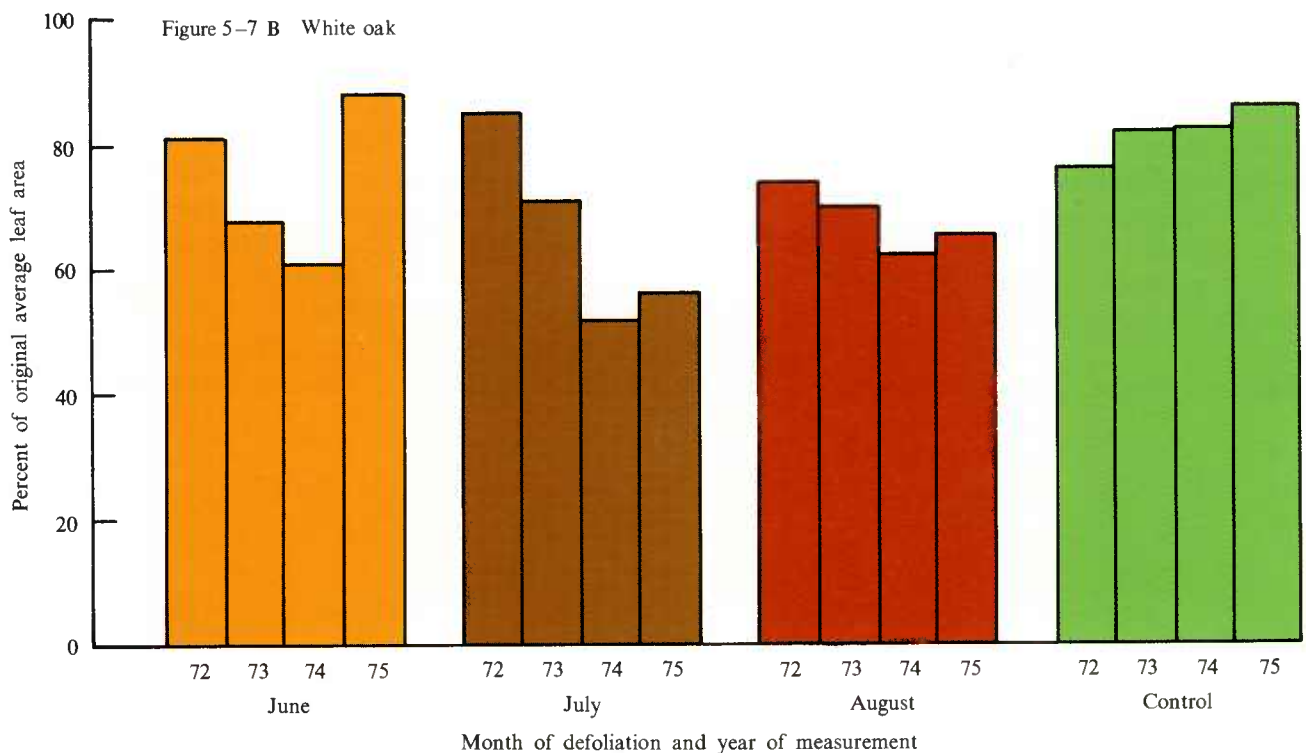
third defoliation than they were after the first defoliation. For black oak and sugar maple defoliated in May, June and July, there was a significant (P=0.05) linear decrease in leaf area. In all three species, leaf area of the trees defoliated in June was smallest after the first year of defoliation (P=0.05). However, subsequent decreases were greater for trees defoliated in May and July.

Total Canopy Area

Repeated defoliation diminishes the total canopy area of the primary leaf system. In red oaks and red maples defoliated completely for 3 successive years, a 45- and 50-percent decrease, respectively, in total area resulted from the combination of decrease in leaf size and numbers (Heichel and Turner 1976). Less severe

defoliation had less effect on total canopy area; in fact, some treatments actually increased total canopy area above that prior to the first defoliation.

In the program study, only complete defoliation was imposed, but it caused similar effects. The effect of defoliation was measured by the total number of leaf clusters per tree, not number of leaves per tree. The average number of leaf clusters per tree was significantly reduced by all defoliations of black oak, white oak, and sugar maple (fig. 5–9, *A, B,* and *C*). In spring 1974, after 3 successive years of defoliation, the average number of leaf clusters in all black oaks, in white oaks defoliated in July, and in sugar maples defoliated in May, June, or July was less than 40 percent of the original number of clusters. All treatments but May-defoliated white oaks and

Figure 5-7 C Sugar maple

Month of defoliation and year of measurement
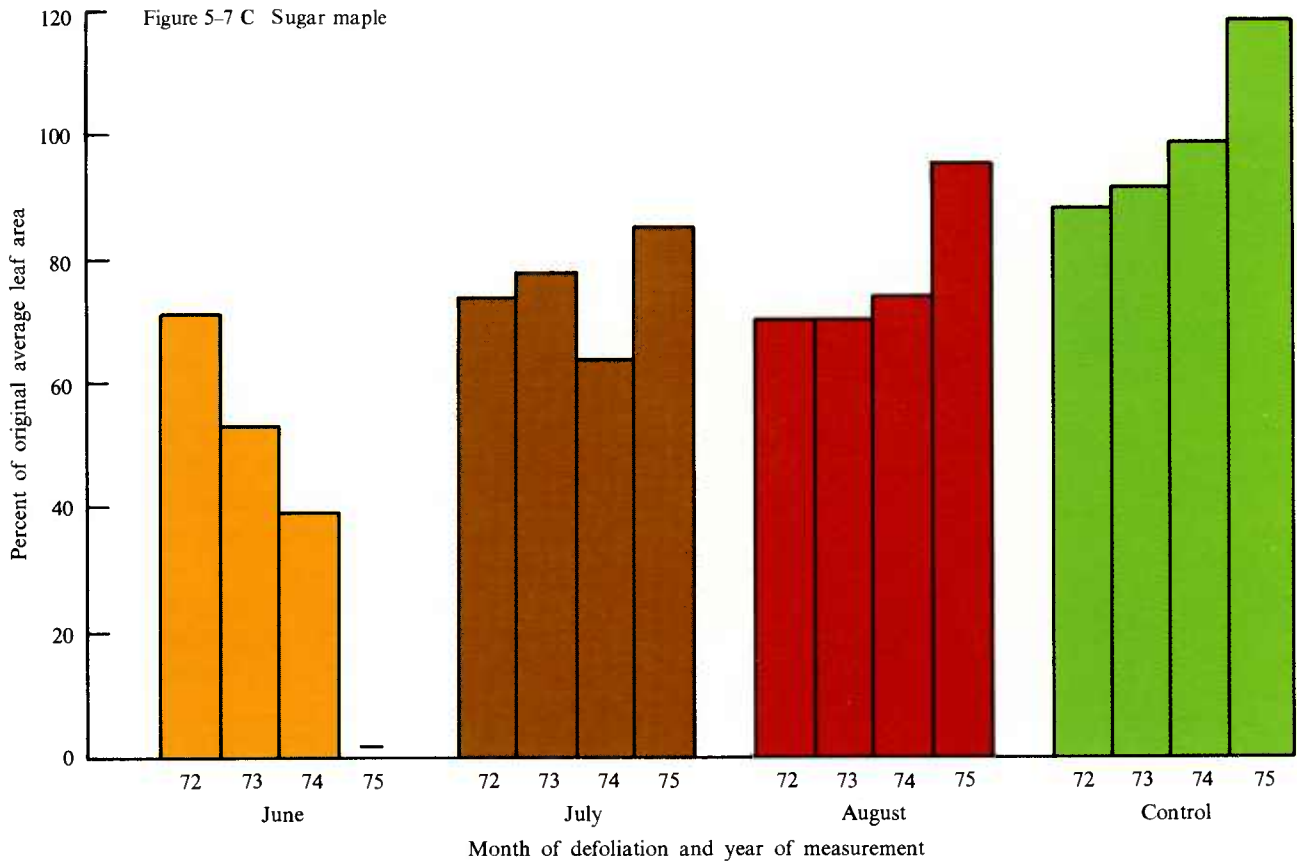
August-defoliated sugar maple had less than 50 percent of their original number of leaf clusters in 1974, and all defoliated trees had less than 60 percent. Disturbance of the control trees was also indicated by a slight reduction in average number of leaf clusters.

In sugar maples the effects of defoliation were progressive for all defoliation times and most severe in June-defoliated trees. In the oaks, the effects were progressive only in white oaks defoliated in July and August. Among the other defoliation times, a large decrease in the number of leaf clusters occurred after the third defoliation. July defoliation had the greatest effect on white oaks; among the black oaks, all treatments were similar.

Twig death accompanied the decrease in number of leaf clusters and accounted for the drastic reduction in the average number of leaf clusters per tree. Although sprouting occurred, the number of sprouts produced in response to twig dieback did not compensate for the loss in leaf clusters.

Once defoliation ceased, some recovery in the average number of leaf clusters occurred. Only in
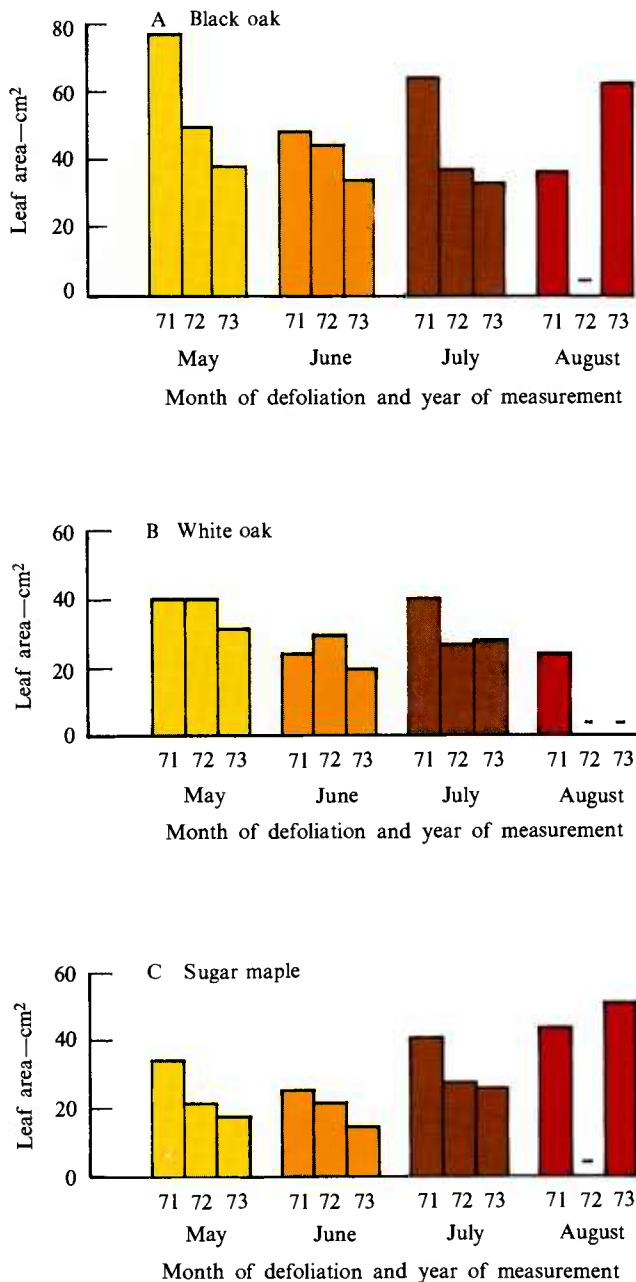
Figure 5-8.—*Change in average area of individual secondary leaves produced after artificial defoliation (100 percent) in May, June, July and August in 1971, 1972, and 1973: A, Black oak—August measurements based on 2 trees in 1971 and 2 trees in 1973; B, white oak—August measurements based on 8 trees in 1971; C, sugar maple—August measurements based on 7 trees in 1971 and 2 trees in 1973.*

May-defoliated black oaks was there a substantial decrease in leaf cluster number after defoliation ceased. In most other treatments, only very slight decreases occurred; in some treatments an increase occurred, but these changes were not significant.

These results show that severe defoliation followed by refoliation has an adverse effect on tree growth not only because the primary food-producing system is removed but also because the area of replacement leaves (secondary) is significantly smaller. This defoliation-refoliation process also alters the tree physiologically (Wargo 1972, Wargo et al. 1972): Growth regulators that control bud dormancy are changed when the leaves are removed; the tree metabolizes reserve foods (primarily starch in deciduous trees) to maintain its living tissues until the new leaves are producing food; buds formed originally for next year open, and new leaves begin growth about 3 to 4 weeks after the tree is defoliated; and new buds must form again within a shortened growing season.

A refoliated tree is completely out of phase with the season. The tender foliage is formed under midsummer growing conditions, which are not as favorable as those in spring. Day length is declining, it is usually hotter and drier, and the growing season is shortened.

This "spring again" condition can have drastic effects on a tree by autumn. Food reserves that are needed to maintain living tissues during the dormant season may be low. Tree tissues may be chemically and physically immature at the onset of the dormant season and may die during the winter.

A defoliated/refoliated tree also starts the following season with fewer, smaller, and possibly less productive leaves (Heichel and Turner 1976), lower food reserves, and mineral imbalances. The tree is more vulnerable to the effects of additional defoliation next season and to attacks by secondary organisms to which the tree is normally resistant.

This effect can be progressive, and successive defoliations can further reduce primary and secondary leaf areas and total numbers. The recovery or
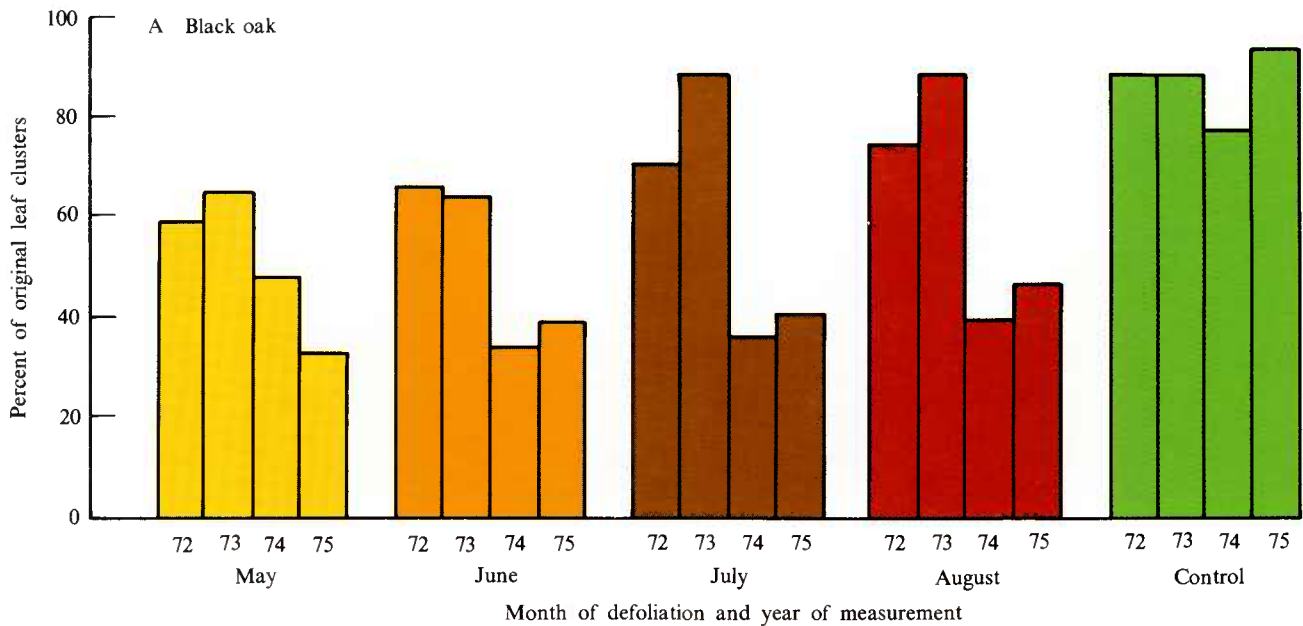
Figure 5-9.—Change in the average number of leaf clusters per tree after artificial defoliation (100 percent) in May, June, July and August in 1971, 1972 and 1973 plotted as percent of the average number of original leaf clusters in 1971 prior to the first defoliation: A, Black oak—average number of leaf clusters in 1971 was 276 for May defoliated trees, 199 for June trees, 325 for July trees, 272 for August trees, and 262 for control trees; B, white oak—average number of leaf clusters in 1971 was 449 for May defoliated trees, 387 for June trees, 521 for July trees, 370 for August trees, and 297 for control trees; C, sugar maple—average number of leaf clusters in 1971 was 615 for May defoliated trees, 369 for June trees, 388 for July trees, 563 for August trees, and 450 for control trees.

stabilization of leaf area and number in the years immediately after defoliation ceased indicates that the progressive deterioration resulted from each additional defoliation. This progressive effect was not observed in similar studies on forest trees (Heichel and Turner 1976). Heichel and Turner (1976) observed that repeated defoliation had little additional effect on leaf area and that the initial defoliation exerted the greatest impact. In the gypsy moth program study, the initial defoliation exerted the greatest effect on leaf area only on trees defoliated in August. Trees used in the Heichel and Turner study were growing in open stands established on former arable land that was not a typical forest site. The absence of mortality in their study even after 3 successive years of defoliation suggests that this was an abnormal situation. Parker and Houston (1971) also observed no mortality after 3 years of defoliation of sugar maples growing on former arable land. The absence of mortality on both sites could be due to the lack of secondary organisms, while the limited effect on leaf area in the Heichel and Turner study could be related to higher fertility in the arable soil.

Effects on Wood Production

Most work on the effects of defoliation and wood production has been done with conifers, especially the spruce budworm/spruce fir complex (Mott et al. 1957, Williams 1967), and the major effects of defoliation on growth of deciduous trees were adequately reviewed by Kulman (1971). This program

Figure 5-9 **B** White oak

Month of defoliation and year of measurement

Figure 5-9 **C** Sugar maple

Month of defoliation and year of measurement
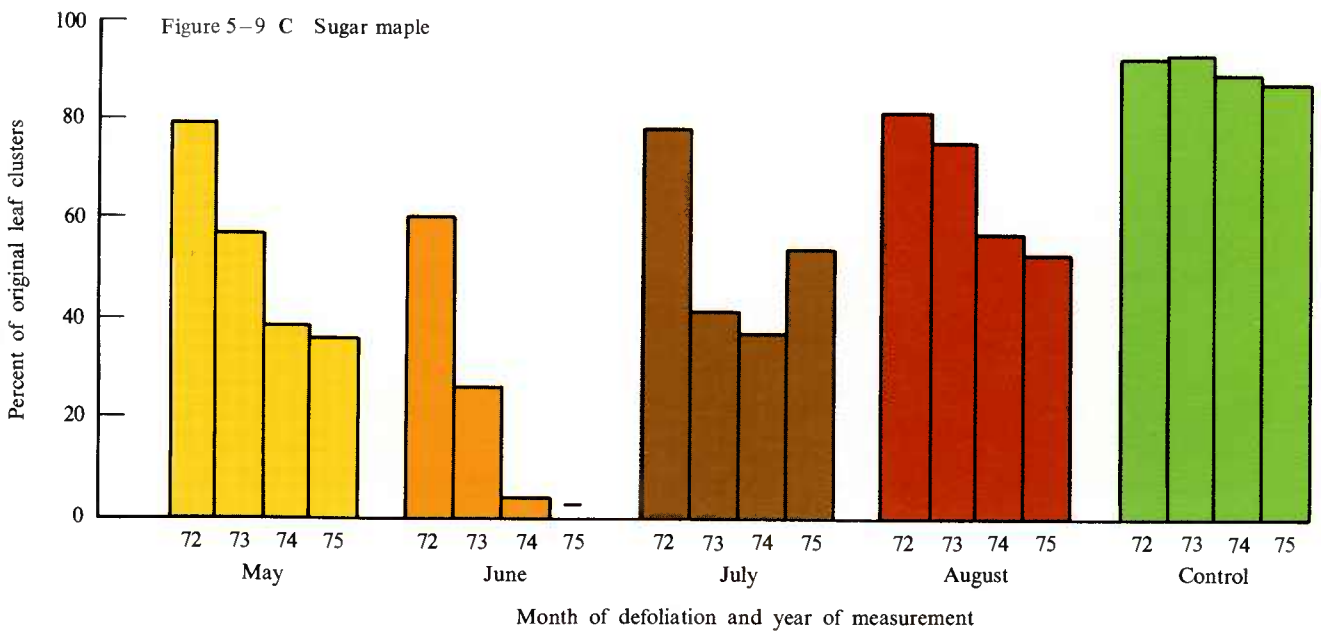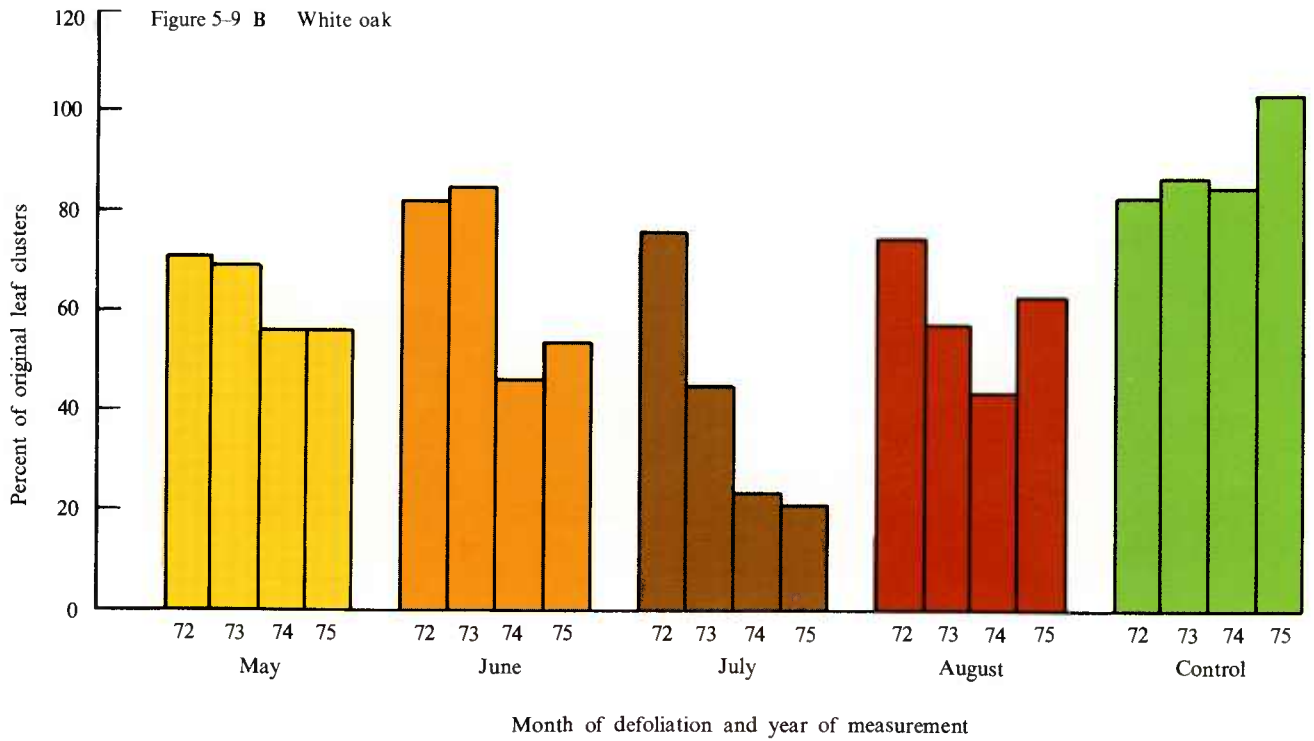
study on small trees provided an opportunity to observe the effects of defoliation at different times during the growing season on radial and terminal growth.

Radial Growth

A 16-cm section of stem at 1.4 m was cut from all trees that died during the 5-year study and from all surviving trees at the end of the study. Discs cut from the opposite ends of the section were sanded and polished. Radial growth was measured with a dissecting microscope fitted with an ocular micrometer. Maximum and minimum increments were recorded for each year on each disc and an average determined for each year. Growth in 1969 and 1970 was averaged and used as the basis for determining change in radial growth.

Radial growth in black oak, white oak, and sugar maple was significantly reduced ($P=0.05$) by all defoliation treatments (fig. 5–10, *A, B,* and *C*). The effect

occurred in the first year of defoliation in all trees but sugar maples defoliated in August. Defoliation in May or June caused the greatest reduction in radial growth in the first year in all three species (table 5–2). In the white oak trees, some reduction in radial growth occurred in 1971 in the undefoliated controls (fig. 5–10, *A, B,* and *C*), Natural populations of gypsy moth and elm spanworm in these stands caused some defoliation of these trees in 1971. After control trees were sprayed in 1972 and 1973 to prevent a similar occurrence, growth rates recovered.

In all three species a progressive decline occurred in radial growth with each year of defoliation (fig. 5–10, *A, B,* and *C*). Minimum growth occurred usually in the third year of defoliation. Once defoliation ceased, trees in some treatments showed some recovery in growth rate. Recovery occurred in black oak defoliated in August, white oak defoliated in May, and sugar maple defoliated in May and August. Even after two growing seasons without defoliation,
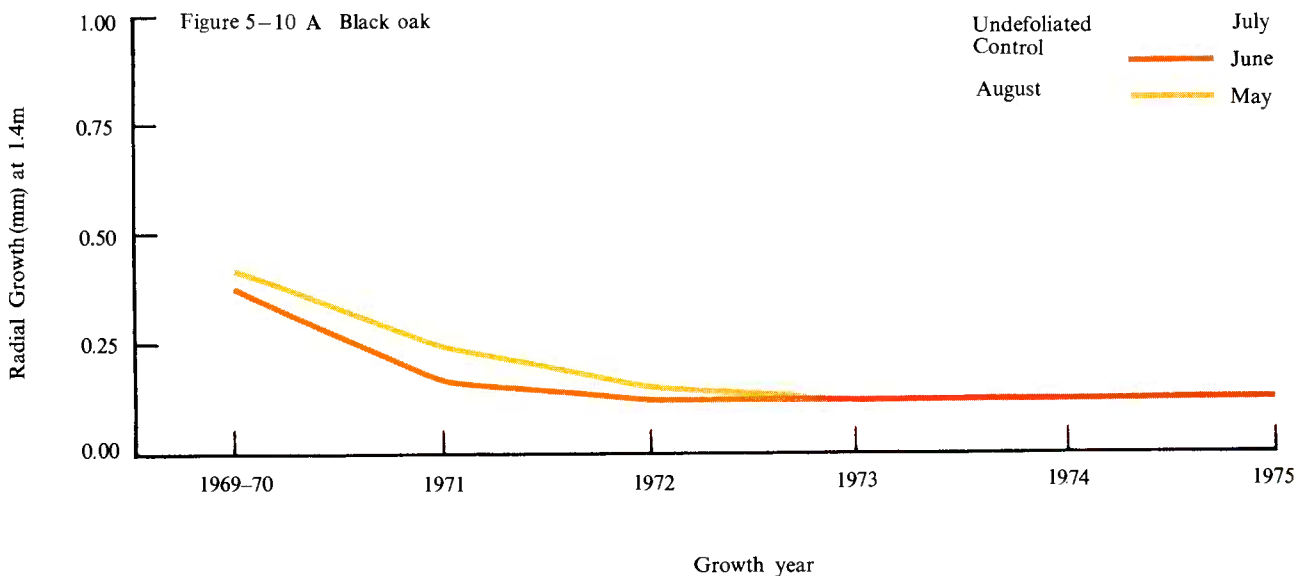
Figure 5–10.—*Effects of artificial defoliation (needed before 100 percent) in May, June, July, or August in 1971, 1972, and 1973 on radial growth at 1.4 m above ground: A, Black oak; B, white oak; C, sugar maple.*

Figure 5-10 B White oak

Figure 5-10 C Sugar maple
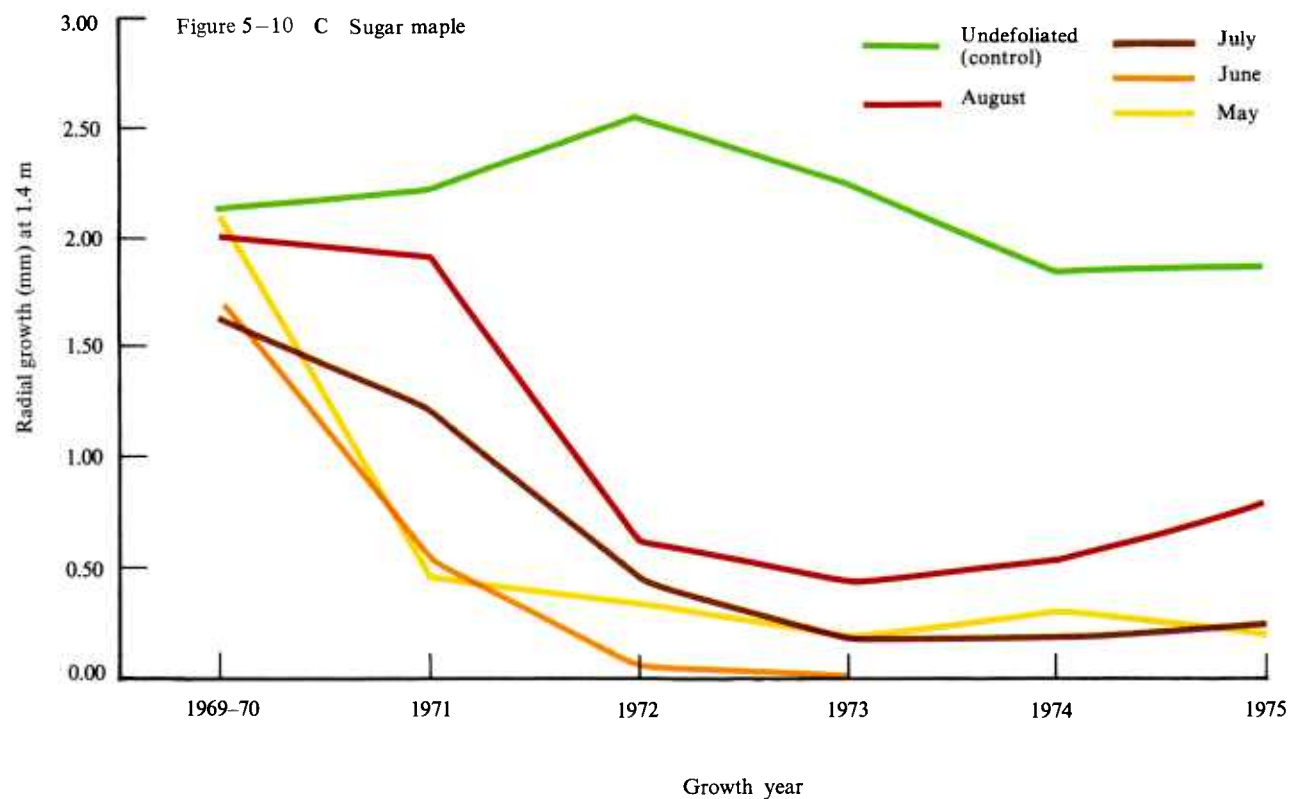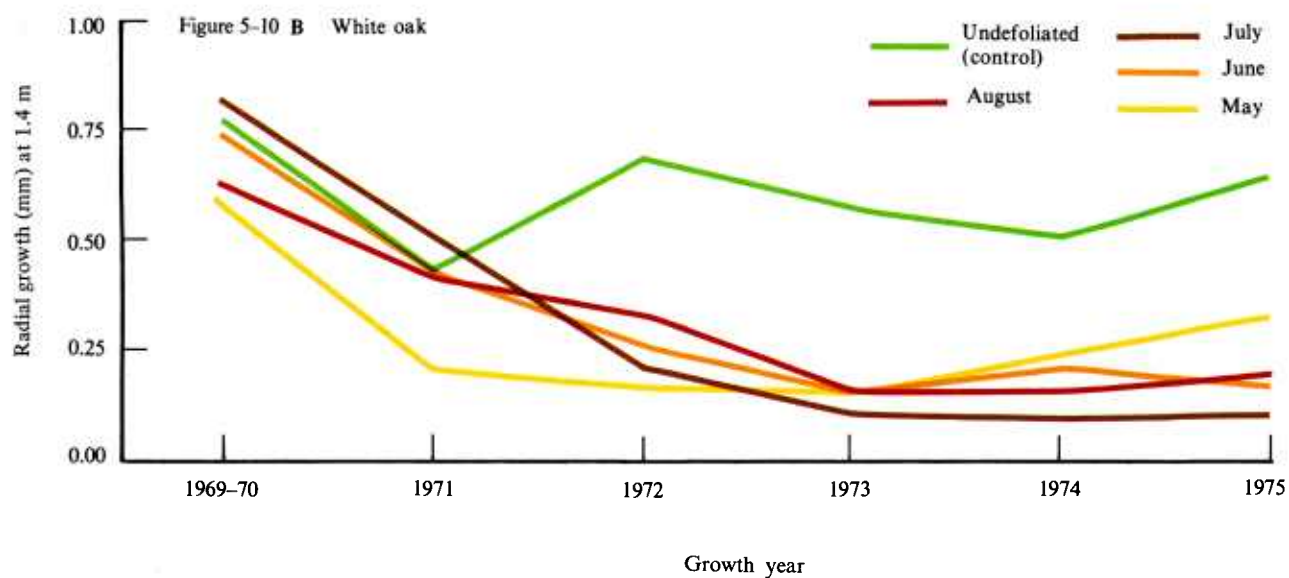
Table 5-2.—*Percent reduction of radial growth in black oak, white oak, and sugar maple after 1 year of artificial defoliation*[1]

Species	Percent reduction, month defoliated			
	May	June	July	August
Black oak	41	57	26	35
White oak	64	45	38	35
Sugar maple	77	70	25	5

[1]Percent reduction based on radial growth averaged for 1969 and 1970

however, only average radial growth of black oaks defoliated in August equaled that of the controls. All other treatments were at best only 50 percent of the original growth increment in 1969 and 1970.

On some surviving sugar maples, no measurable growth occurred at 1.4 m for 1 to 3 years. On several trees, growth recurred at 1.4 m in the two growing seasons with no defoliation. Some measurable growth occurred on all the oaks; at least the early wood vessels and some late wood cells were produced.

In another study on larger trees defoliated by the gypsy moth, growth rate was measured at 1.4 m by taking increment cores and measuring the width of the annual rings with a dendrochronometer.

Like the artificial defoliation study, insect defoliation decreased the annual increment and successive defoliations caused additional decreases in the growth rate (table 5-3). In contrast to the artificial defoliation study, the reduction was less in the naturally defoliated trees, and trees recovered more rapidly, especially the red oaks. These contrasts emphasize the difference between natural progressive defoliation that occurs over a 4- to 6-week period and the sudden artificial defoliation that occurs in 1 day. It points out the dangers of applying data from artificial defoliations studies to field situations.

Defoliation by the gypsy moth and other defoliators can obviously cause significant reduction in the growth of trees beginning in the early stages of insect population buildup and lasting for several years after defoliation ceases. While not a serious loss in the relatively unmanaged forests of the Northeast, this

effect on growth rate does represent potential economic losses in areas where oaks and other hardwoods are managed more intensively. It can mean delayed rotations or substantial loss of wood where rotations cannot be delayed. This fact emphasizes the need for a comprehensive growth model for deciduous trees; such a model is presented and discussed in chapter 3.

Terminal Growth

Defoliation has a significant effect on terminal growth. In the program study on small trees, terminal growth was determined on all surviving trees. Unfortunately, it was not planned as part of the original study and no markers were placed on the trees in 1971 from which to determine subsequent growth. Because of refoliation, it was difficult to determine increment of growth for each year based on budscale scars and consequently only the data of 1973-75 were meaningful.

Terminal growth of all defoliated trees was significantly less than growth of the control trees in sugar maple and white oak (table 5-4). In black oak, August defoliated trees had growth increments similar to the controls, but the other defoliated trees had significantly less growth. Either August defoliation had no effect on terminal growth of the black oak or terminal growth prior to defoliation was much greater than that of the controls. In all three species, the late defoliations had less effect than early ones on terminal growth. All trees showed signs of terminal growth rate recovery in 1975 but were still growing less than the control trees.

In older stands where dominance has been established, this terminal growth effect is probably of limited consequence. However, in young stands of mixed hardwoods where preferential defoliation occurs, significant reduction of terminal growth could result in permanent changes of canopy positions.

Effects on Food Reserves (Starch)

As indicated in the beginning of this section on individual tree relationships, defoliation induces

Table 5-3.—*Annual radial increment of growth in red and white oaks prior to and after heavy defoliation by the gypsy moth in 1971-73*[1]

		Percent defoliation		Annual radial increment (mm)					
Tree species	Number of trees	1972	1973	1970	1971	1972	1973	1974	1975
Red oak	79	90	65	1.2	1.0	0.7	0.6	0.9	1.2
White oak	43	100	85	1.1	1.0	.7	.6	.6	.8

[1]Actual defoliation in 1971 was not estimated.

Table 5-4.—*Terminal growth on black oaks, white oaks, and sugar maples that were artificially defoliated in 1971, 1972 and 1973*

			Growth (cm)		
Tree species	Month defoliated	Number of trees	1973	1974	1975
Black oak	May	5	1.9	2.3	3.2
	June	1	1.6	1.6	3.7
	July	7	2.0	1.4	2.0
	August	7	6.8	6.1	6.7
	Control	10	8.1	6.7	6.8
White oak	May	5	3.0	4.0	4.2
	June	6	1.9	2.6	3.3
	July	5	3.1	3.2	3.7
	August	8	6.0	5.9	6.6
	Control	10	10.4	11.8	11.4
Sugar maple	May	6	2.6	4.0	6.2
	June	0	—	—	—
	July	7	3.0	7.5	6.2
	August	10	5.0	7.3	17.8
	Control	10	39.0	38.7	28.5

numerous chemical changes in the tree. One of the major changes that occurs is the mobilization of food reserves to support the living tissue in the absence of leaves.

Light to moderate defoliation for 1 year seems to have little significant effect on food reserve storage, as indicated by the starch content of the roots (Wargo et al. 1972). In such situations, starch reserves are probably not mobilized. Slightly lower starch occurs because of reduced photosynthesis and not as much is available for storage.

Work on sugar maple indicates that the greatest effect on starch reserve occurs when defoliation is heavy enough to cause refoliation of the tree in the same season (Wargo et al. 1972). When defoliation is severe, little to no carbohydrate is produced, and the tree must mobilize stored foods to maintain its actively respiring tissues. The decline of starch content, however, is apparently not just a response to lack of food. Defoliated-refoliated sugar maples mobilize more starch in the roots than girdled or cutoff trees (Parker 1974), indicating that the process

of refoliation places an additional demand on or causes greater mobilization of stored food.

The effect on starch occurs rapidly and in defoliated sugar maples is evident at least 2 weeks after complete defoliation (Wargo 1972). Final starch content is related to the initial content—the higher the starch content of the tree when defoliated, the higher the starch content remaining in the tissues. Since food reserves are important to tree survival, starch content after defoliation is important and may be a key to tree survival after defoliation. Staley (1965) in his study on decline and mortality of oak after defoliation by leaf rollers found diminished starch reserves in the trees most likely to die after defoliation. The relationship of starch content and mortality is discussed in Death of Trees, this chapter.

To acquire additional information on the effects of defoliation on food reserves, starch content of the twigs and roots of the small trees (described in Defoliation and Tree Growth, this chapter) was determined. Starch content of the roots was determined by chemical extraction and colormetric measurement (Hassid and Neufeld 1964, Siminovitch et al. 1953) and histochemically by staining cross sections with I_2KI solution (Wargo 1975a). Root starch was measured on all trees at the time of defoliation in 1971, on all surviving trees each autumn after defoliation in 1971, 1972, and 1973, and after no defoliation in 1974 and 1975. Only a single root sample was taken each time to minimize damage to the roots. Twig starch was determined by the chemical extraction technique. It was measured at the time of defoliation and in each autumn in 1971 and 1972. Twig sampling was discontinued when considerable dieback occurred on the trees.

Starch content of the roots of all defoliated trees was significantly lowered in all three species (table 5-5). The effect was immediate for most defoliation treatments. In autumn 1971, after the first defoliation only black oaks defoliated in August, white oaks defoliated in May or June, and sugar maples defoliated in August were not significantly lower in starch than the undefoliated control trees. The differences among defoliation treatments were partially related to the

initial starch content at the time of defoliation. Trees defoliated later in the year had higher starch content than some earlier defoliated trees and hence had more starch at the end of the first year. This confirmed earlier observations on initial starch content and the effects of defoliation (Wargo 1972).

By autumn 1973, after three defoliations, all defoliated trees were significantly lower in starch content than undefoliated trees. Starch content of surviving defoliated trees began to recover in the first growing season without defoliation. In autumn 1974, starch content was significantly better than in autumn 1973 in all but white oak and sugar maple defoliated in July. Additional recovery occurred in 1975, but starch content was still lower than in the undefoliated trees.

Except for two black oaks, one defoliated in May and one in June, that had starch contents at least five times lower than the average tree when defoliated, and three sugar maples, one defoliated in May and two defoliated in June, most trees tolerated at least 2 years of defoliation before root-starch content was depleted (table 5-6). Starch content was depleted at some time during the 5-year study in 28 black oaks, 25 white oaks, and 19 sugar maples out of 40 defoliated trees for each group. In sugar maple, most of the 19 trees that had depleted starch were depleted by autumn 1972 after the second defoliation. In both oak groups, most of the trees were depleted only after the third defoliation.

Defoliation also had an effect on twig starch content, but it was not nearly as severe as in the roots (table 5-7). The dominant negative effect occurred in the later defoliations, especially August in the oaks. The effects of the second defoliation was evident primarily among the sugar maples; their twig starch content in the autumn after the second defoliation was approximately half of what it was the first autumn.

The seasonal pattern of starch storage was similar in the roots and twigs for all three species. The seasonal pattern can be determined from tables 5-5 and 5-7 starting with initial starch content of the May through August defoliated trees and finishing with the autumn 1971 starch content of the control trees. In sugar maple, starch content was lowest in May and

Table 5-5.—*Average starch content in roots of black oak, white oak, and sugar maple before and after 3 years of artificial defoliation in May, June, July, or August, 1971-73*

| Tree species | Month defoliated | Initial starch 1971[1] | Percent autumn starch content | | | | |
			1971	1972	1973	1974	1975
Black oak	Undefoliated (control)	14.7	24.0	27.3	15.5	15.5	25.6
	May	16.2	11.1	2.2(9)[2]	.1(7)	3.7(5)	18.6(3)
	June	10.6	8.3	2.0(8)	0 (7)	3.7(5)	18.6(3)
	July	22.0	10.7	2.8	.8	4.2(8)	8.8(6)
	August	23.4	17.7	7.5	2.7	8.9(7)	15.5(6)
White oak	Undefoliated (control)	15.1	13.3	19.3	13.1	20.0	19.3
	May	13.7	10.6	4.7	4.0	11.3(6)	16.5(4)
	June	5.8	13.3	10.2	2.0	7.2(6)	10.2(6)
	July	9.5	4.3	1.5	.2	.6(6)	.7(4)
	August	13.8	4.4	4.1	1.2	11.0(9)	13.2(8)
Sugar maple	Undefoliated (control)	2.0	18.0	15.0	9.4	9.9	8.1
	May	2.4	16.0	7.2(9)	4.6(7)	7.4(7)	6.8(7)
	June	5.8	.9	.1(8)	.3(3)	—	—
	July	10.8	3.6	1.1	2.0	2.4(7)	3.6(7)
	August	10.1	3.8	5.5	3.6	6.1	6.2

[1]Starch content on a percent dry weight basis at the time of defoliation and for undefoliated controls in May 1971.

[2]Numbers in parenthesis indicate the number of surviving trees used to determine the average; indicated only where fewer than the 10 original trees.

Table 5-6.—*Total number of trees that had depleted root starch, by year in which depletion first occurred, for black oak, white oak, and sugar maple trees artificially defoliated for 3 years, 1971-73*

| Tree species | Number of trees with depleted starch | Number of trees and autumn when starch was first depleted | | |
		1971	1972	1973
Black oak	28	2	6	20
White oak	25	0	7	18
Sugar maple	19	3	12	4

progressively increased each month through autumn. In the oaks, starch content was high in May, decreased to the low in June, and progressively increased each month through autumn.

The different patterns in seasonal changes indicate the importance of knowing normal seasonal patterns. For example, in May, starch is mobilized normally in sugar maple, and low starch content of roots of sugar maple in May will not necessarily indicate if a tree has been defoliated. In contrast, both oak species were normally high in starch in May. It also points out that because of seasonal changes, starch content is not as stable during the growing season as it is in autumn. Thus, for determining the effects of defoliation, autumn starch offers the advantage of being normally high in most deciduous species and stable.

Defoliation, Dieback, and Mortality

Philip M. Wargo

Dieback of Crowns

Dieback of twigs in the upper crown of trees is one of the first symptoms of the effects of defoliation, but it is not peculiar to defoliation. Dieback of the crown can result from numerous causes and is considered a

Table 5-7.—*Average starch content of the twigs of black oak, white oak, and sugar maple before and after artificial defoliation in May, June, July, and August of 1971 and 1972.*

| Tree species | Defoliated | Starch content[1] | | | |
| | | 1971 | | 1972 | |
		Initial[2]	Autumn	Initial[2]	Autumn
Black oak	May	2.9	5.4	0.8(9)[3]	4.3(9)
	June	1.4	5.8	1.4(9)	5.2(8)
	July	2.4	5.4	3.2	4.5
	August	4.4	3.2	5.3	2.8
	Control	—	5.0	—	5.2
White oak	May	6.3	7.3	1.6	6.5
	June	1.6	7.6	1.1	6.2
	July	1.5	5.5	1.8	4.5
	August	3.6	3.8	3.8	2.3
	Control	—	7.7	—	6.1
Sugar maple	May	.5	3.7	.4	1.8
	June	1.5	2.0	.8(9)	1.4(8)
	July	2.4	3.2	2.4	1.5
	August	3.1	2.2	4.1	1.2
	Control	—	2.9	—	2.5

[1]Determined as a percent of dry weight.
[2]Starch content determined at time of defoliation.
[3]Numbers in parenthesis indicate the number of surviving trees used to determine the average and is indicated only when fewer than the 10 original trees.

general symptom of decline diseases of forest trees (Houston 1973).

Defoliation can cause rapid and significant crown dieback in a relatively short time and is accompanied by significant sprouting from adventitious and latent buds. In fact, some trees can be reduced to living sprouts on the lower portion of the main stem in just 2 years. This has been observed in both natural and artificial defoliation studies (Giese et al. 1964a,b, Staley 1965, Nichols 1968).

Dieback of the twigs and branches seems to be affected by three factors: Time of season of defoliation, severity of defoliation, and whether the tree refoliates or not. In a comprehensive study on the genesis of maple blight, terminal mortality was found to be greater on trees sustaining a greater percentage of crown defoliation and was found to decrease as the time of defoliation became later in the growing

season, especially from mid-August on (Giese et al. 1964b). Also, in similar studies on sugar maple, less dieback was observed with late-season defoliation, probably because refoliation did not occur (Wargo and Houston 1974). Terminal mortality is probably related to insufficient amounts of food reserves and to the failure of the new buds formed after refoliation to mature and harden properly prior to the onset of winter, because of a shortened growing season.

Dieback is progressive and crown condition deteriorates with each succeeding defoliation. Crown-condition changes of the small treees used in this study (described in Defoliation and Tree Growth, this chapter) occurred after a single defoliation for all defoliation treatments, including late-season (mid-August) defoliation (table 5-8). After the third year of defoliation, the average defoliated tree had less than 50 percent of its original live crown.

Table 5-8.—*Average crown condition in the spring of each year for black oak, white oak, and sugar maple trees before, during, and after artificial defoliation in May, June, July, or August, 1971-73*

Tree species	Defoliation treatment	Crown condition[1]				
		1971	1972	1973	1974	1975
Black oak	Undefoliated (control)	[2]1.6	1.6	1.6	1.7	1.7
	May	1.9	2.8(9)	2.9(9)	3.2(5)	3.8(5)
	June	2.0	2.8(9)	2.7(8)	3.5(2)	3.0(1)
	July	1.4	2.2	2.1	3.2	3.3(8)
	August	1.5	2.2	2.3	3.0(8)	3.1(7)
White oak	Undefoliated (control)	1.4	1.2	1.4	1.3	1.4
	May	1.5	2.1	2.2	2.9(8)	2.8(6)
	June	1.1	1.4	1.9	2.3(6)	2.3(6)
	July	1.1	1.6	2.8	3.7(6)	3.8(6)
	August	1.5	2.0	2.7	3.0(9)	2.8(9)
Sugar maple	Undefoliated (control)	1.1	1.1	1.1	1.1	1.1
	May	1.3	1.4	2.3(9)	2.8(7)	2.8(6)
	June	1.1	2.3(9)	3.8(5)	4.0(1)	—
	July	1.2	1.7	2.6	1.8	2.3(7)
	August	1.1	1.4	1.9	2.3	2.3

[1]Crown condition determined in mid-May of 1971 for all trees; $n=10$ for each treatment-species combination. Crown condition in subsequent years is based on trees alive in the spring of each year. Number in parenthesis indicates number of live trees when fewer than 10.

[2]Crown numerically rated on basis of percent of dead wood in crown; $1 < 25$ percent, $2 = 25$-50 percent, $3 = 50$-75 percent, $4 > 75$ percent, $5 = 100$ percent.

In white oak, trees defoliated in July suffered the greatest amount of dieback, and in sugar maple, defoliation in June resulted in the greatest crown damage. All defoliation treatments caused similar crown damage in the black oak; overall they sustained the greatest amount of crown dieback (table 5-8), which may have been because they had, on the average, the poorest crowns at the start of the study.

This relationship of crown condition prior to defoliation and subsequent damage by defoliation has also been reported for natural defoliation studies. Based on historical data from the Melrose Highlands Gypsy Moth Laboratory in Massachusetts, Campbell and Valentine (1972) developed tables for determining crown condition changes based on species, dominance class, diameter class, defoliation history, and crown condition prior to defoliation. The tables indicate that trees with good crown conditions prior

to defoliation tend to deteriorate less rapidly and not as much than trees with only fair crowns. The difference becomes less as the severity and frequency of defoliation increase.

There is also a relationship of crown dominance and crown dieback. Again analysis of data generated by the Melrose Highlands laboratory showed that trees with subdominant crowns tended to deteriorate the most over a 10-year period in which heavy defoliation occurred several times (Campbell and Sloan 1977).

Trees can recover. In response to dead twigs and branches, latent and adventitious buds sprout and form a canopy of leaves within the deteriorating tree crown (Giese et al. 1964a). In the absence of additional defoliation, the new shoots continue growing, the dead twigs and branches are shed, and the trees recover. This process may take as many as 10 years.

Campbell and Sloan (1977) observed that after a single heavy defoliation, the number of good crown trees declined over a period of 5 years and it was another 5 years before the number of good crowned trees returned to their condition prior to the defoliation. Obviously, if defoliation is repeated, the amount of deterioration will increase and hence the length of time to recover.

Death of Trees

Physiological Condition

Mortality of trees in poor physiological condition (low vigor) tends to be greater and faster after defoliation. Campbell and Sloan (1977), in analyzing data from the Melrose Highlands laboratory on gypsy moth defoliation, found that mortality after defoliation was highest in trees with poor crowns, lowest in trees with good crowns, and intermediate in trees with fair crowns. Crown condition is a general expression of the physiological condition of the trees. The actual number of trees that died in each group depended on the severity and frequency of defoliation, but the pattern of lower mortality in the good-crown trees held.

Tree dominance, another expression of vigor but less dynamic than crown condition, also influences mortality rates (Campbell and Sloan 1977). The Melrose data showed that mortality was higher in the subdominant trees. There was also an interaction of dominance and crown condition, and mortality increased as the crown position and crown condition decreased.

The relationship of high mortality and poor crown condition has also been observed in recent outbreaks of the gypsy moth. Gansner and others (1978) observed that mortality in plots in Pennsylvania after gypsy moth defoliation was higher on those plots having higher numbers of trees with poor crown conditions.

Whether higher mortality is related to only poor crown condition is not certain. There is a possibility that the factor(s) leading to the poor crown condition

also plays a role. Wargo (1977) observed that trees may be predisposed to the effects of gypsy moth defoliation by previous defoliation, in this case previous defoliation by the oak leaf roller. In the Melrose Highlands data, trees with the poorer crown conditions may have been in this condition from previous gypsy moth defoliation. The same may be true of the recent Pennsylvania experience (Gansner et al. 1978).

Mortality is not limited to the obviously weakened or less vigorous trees. Many trees that look healthy die, and some healthy-looking trees next to other healthy-looking trees die. Obviously crown condition does not always accurately reflect a tree's physiological condition.

Studies on root starch content in small, artificially defoliated trees and trees in naturally defoliated forest stands indicate that starch content is a more dynamic indicator of physiological condition than crown condition. The studies also confirm the relationship of higher or faster mortality in trees that are in poor physiological condition.

In the study on small trees that were artificially defoliated, some mortality occurred in each group in each defoliation treatment time except sugar maples defoliated in August (fig. 5-11, A, B, and C). In black oak and sugar maple, mortality was highest in trees defoliated in June. In white oak, mortality was equally high in May and July. Mortality was highest among the black oak trees (24 of 40 trees), lower in white oaks (18 of 40 trees) and lowest in sugar maples (16 of 40 trees). With the exception of white oak and sugar maples defoliated in May, higher mortality occurred in the months with lower initial starch content for each species (tables 5-5 and 5-9).

There was also an association of low starch content and mortality within each defoliation time for each of the species studied (table 5-9). The average initial starch content of trees that died over the duration of the experiment was consistently lower than the initial starch content of the trees that survived. Also, the autumn starch content after each defoliation in most of the species/defoliation time combinations was lower for the trees that died (table 5-9).
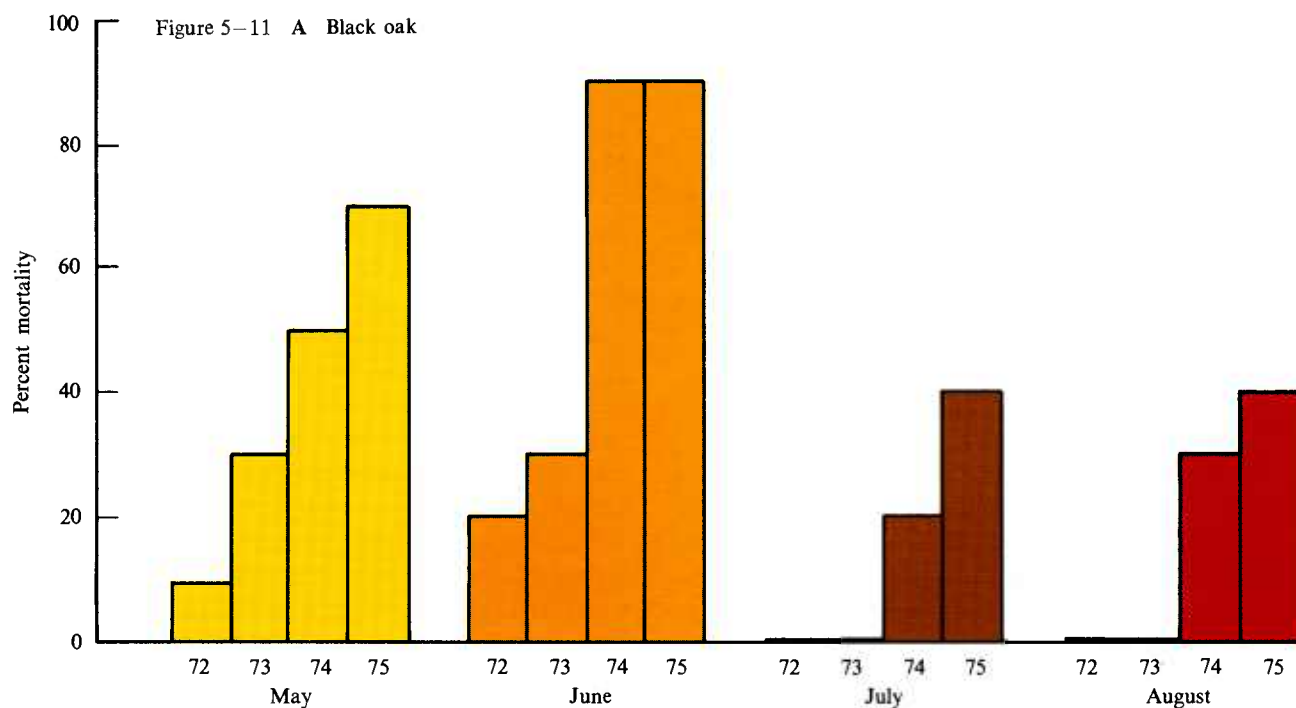
Figure 5–11.—*Cumulative mortality after artificial defoliation (100 percent) in May, June, July or August in 1971, 1972, and 1973:* A, *Black oak;* B, *white oak;* C, *sugar maple.*

Figure 5-11 C Sugar maple
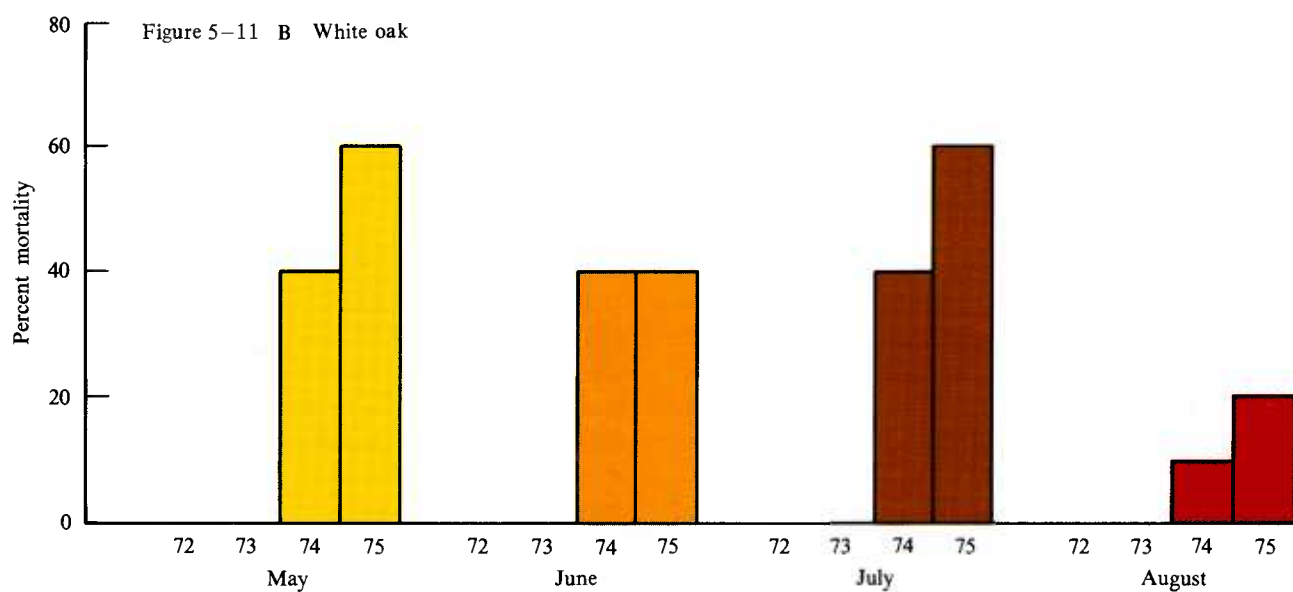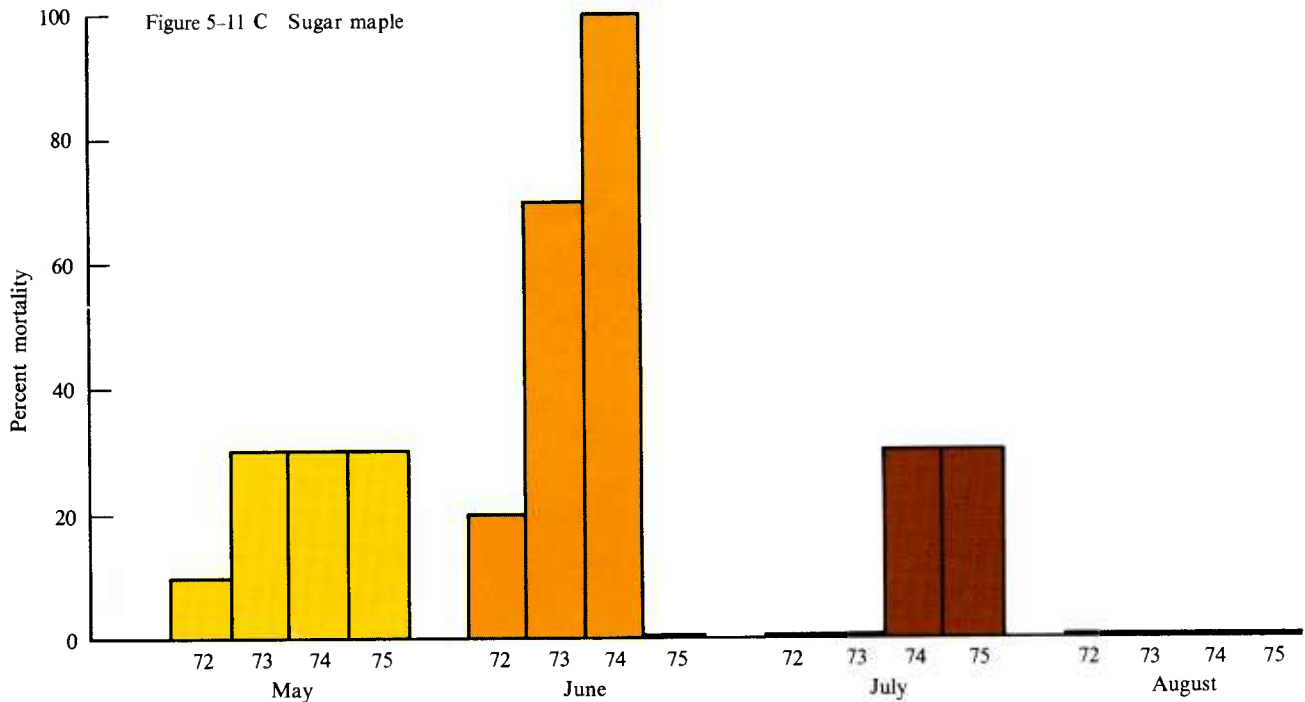
There was also a relationship between starch content and year of death. Trees that died earlier, and hence after fewer defoliations, had on the average lower initial starch contents than trees that died later (fig. 5-12). This relationship was also observed within each species (table 5-10).

Starch content in autumn 1973, after the third consecutive defoliation, seemed to be most critical. Of the 58 trees that died, 32 trees (55 percent) died in 1974, and most of these trees died during the winter (table 5-10). Twenty-seven of these had no starch in autumn 1973, and the other five had less than 1 percent starch at that time. Some trees that had no starch in autumn 1973 survived, but no tree that had 2 or more percent starch in the roots in 1973 died. All 10 sugar maples and five of the six black oaks that died in 1972 and 1973 had no starch in the autumn prior to death.

In describing characteristics of declining scarlet oak after defoliation by oak leaf roller, Staley (1965) also reported the absence of starch in trees that were dying and low starch in trees most likely to die.

Time of Defoliation

Mortality also depends on when during the growing season the tree is defoliated. For example, in the artificial defoliation study on small trees described in Defoliation and Tree Growth—Primary Leaves, repeated defoliation in August had much less effect on trees than earlier defoliations primarily because the trees did not refoliate. This occurred in each of the three species observed. There were also differences in the effects among the earlier defoliation treatments in May, June, and July, but there were distinct species differences. For sugar maple, defoliation in June was most severe and resulted in the fastest and greatest damage. All trees were dead by the spring following the third defoliation; 70 percent were dead after two defoliations. May and July defoliations caused much less damage. June defoliation also caused the greatest damage in the black oaks, but the effect of defoliation in May was almost as adverse. Mortality in the white oaks was similar among the early defoliation treatments, but defoliation in July was more adverse,

Table 5-9.—*Relationship of crown condition (alive or dead) by autumn 1975 to average root-starch content of black and white oak and sugar maple trees when artificially defoliated in May, June, July, and August of 1971 and each autumn after defoliation, 1971–73*

Species	Month of defoliation	Crown condition by 1975	Number of trees	Average root-starch content			
				Initial	1971	1972	1973
Black oak	May	Alive	3	17.0	16.0	5.3	0.3
		Dead	7	15.8	9.0	1.0	.0
	June	Alive	1	11.0	9.0	1.0	.0
		Dead	9	10.5	8.8	1.8	.0
	July	Alive	6	23.2	11.0	2.0	1.0
		Dead	4	20.2	10.2	4.0	.5
	August	Alive	6	24.2	19.2	5.8	4.2
		Dead	4	22.2	15.5	10.0	.5
White oak	May	Alive	4	17.2	12.5	10.2	10.0
		Dead	6	11.3	9.3	1.0	.0
	June	Alive	6	6.5	16.8	16.0	3.2
		Dead	4	4.7	8.0	1.5	.2
	July	Alive	4	11.7	5.5	1.7	.2
		Dead	6	6.8	3.5	1.3	.1
	August	Alive	8	15.0	3.6	4.6	1.5
		Dead	2	9.0	7.5	2.0	.0
Sugar maple	May	Alive	7	2.4	20.3	7.8	4.7
		Dead	3	2.3	9.5	6.0	.0
	June	Alive	0	—	—	—	—
		Dead	10	5.8	.9	.1	.3
	July	Alive	7	11.6	4.0	1.1	2.7
		Dead	3	9.0	2.7	1.0	.3
	August	Alive	10	10.1	4.2	5.5	3.7
		Dead	0	—	—	—	—
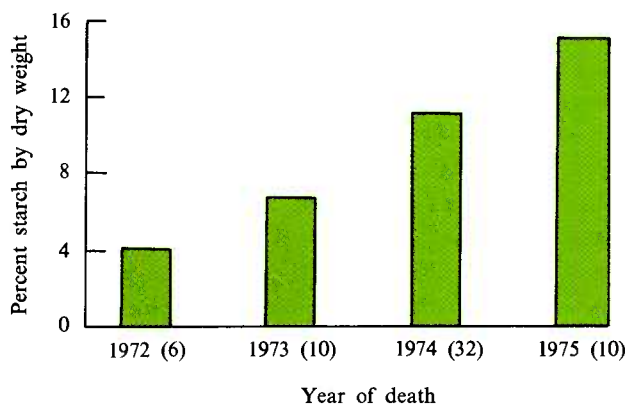
Figure 5-12.—*Average initial starch content in the roots of all trees that died and the year of death. Number in parenthesis beside year of death indicates the number of trees that died each year.*

on the basis of the other parameters used to measure the effects of defoliation.

This difference in timing effects between the two oak species may reflect phenology differences. Leaf development of white oak was behind that of the black oak in mid-May when initial defoliation was imposed. This could account for the different response of the two species to early defoliation. It is possible that stages of growth were still different in mid-July, also resulting in a different response by the two species.

Results from the artificial defoliation studies indicate that defoliation when the leaves are just fully expanded and the tree is growing rapidly is critical. It is at this time that most of the reserve foods have been

Table 5-10.—*Number of trees that died (D) each year and the initial starch content (IS) in 1971 for black oak, white oak, and sugar maple trees artificially defoliated in May, June, July, or August 1971-73*

Number of defoliated trees by species[1]	Year										Total
	1971		1972		1973		1974		1975		
	D	IS	D	IS	D	IS	D	IS	D	IS	
Black oak (40)	0	—	3	5.0	3	14.0	13	16.6	5	20.2	24
White oak (40)	0	—	0	—	0	—	13	6.8	5	10.4	18
Sugar maple (40)	0	—	3	3.3	7	4.1	6	9.1	0	—	16
Total[2]	0	—	6(3)		10(3)		32(22)		10(1)		58(29)

[1]No undefoliated trees died.
[2]Number in parenthesis indicates number of trees that were dead in the spring of that year.

utilized but have not been restored and the leaves are just beginning to become fully functional. Energy demands are greatest because of the newly formed and rapidly growing tissues. Defoliation at this time leaves the tree with no current energy source and little reserve food to meet this demand. Early defoliators such as the gypsy moth consume the major portion of a tree's foliage at this time.

Even early-season defoliators such as leaftiers and leaf rollers can cause severe damage. In many situations the leaves never fully expand but are matted and tied by the larvae and then fed upon. In these cases, the leaves never become fully functional and new buds may not be formed or may be consumed by the larvae. Refoliation is less than normal and the tree is energy deficient. Even late-season defoliation may be harmful; in some cases buds may swell but not break into leaf (Houston and Kuntz 1964) and are susceptible to winter damage from freezing and dessication.

The effect of the timing of defoliation depends on the species, phenology, and the length of the growing season after defoliation ceases. Thus as the gypsy moth moves southward, the effects of defoliation may change because of species and climatological differences. One might speculate that the effect may not be as severe as in the Northeast because of the longer growing season. Artificial defoliation studies can be used to gain some insight into this problem.

Secondary Organisms

Data presented here from the program study on small trees and observations in naturally defoliated areas (Staley 1965) suggest that carbohydrate starvation plays a significant role in mortality of trees after defoliation. Trees very low or depleted of reserves simply starve.

In the small-tree study discussed in the previous section, of the trees that died 50 percent did so during the winter (table 5-10). This could indicate simple starvation. However, the majority (65 percent) of these were also attacked by *Armillaria mellea*, the shoestring fungus, and *Agrilus bilineatus*, the twolined chestnut borer. This plus the fact that many trees having no starch in the roots survived indicate that secondary organisms and not just starvation are involved in mortality of trees after defoliation. In the absence or scarceness of secondary organisms, mortality does not occur. In the Heichel and Turner study (1976) and in a study by Parker and Houston (1971), no mortality was observed on trees that were defoliated for 3 successive years. In both studies trees were growing on former arable soil where the presence

of *A. mellea* would be limited. In the Heichel and Turner study (1976), the presence of *A. bilineatus* was also probably limited because it was on a nonforest site.

In oak mortality after defoliation, the dominant secondary organisms reported have been *A. mellea* and *A. bilineatus* (Staley 1965, Nichols 1968, Kegg 1973, Dunbar and Stephens 1975, Côté 1976, Wargo 1977), but other organisms are probably involved. Staley (1965) found an unidentified fungus that was consistently associated with roots on dead and dying oaks and that was intimately associated with *A. mellea*. *Stegonosporium* species have been linked with twig dieback in maple (Hibben 1964), and *S. ovatum* seemed to play an aggressive role in dieback of stems of artificially defoliated sugar maples (Wargo and Houston 1974).

Defoliation predisposes trees to attack by these organisms. Either the tree becomes more atractive to these secondary organisms and the tree is attacked, or the ability of the tree to resist attack by these organisms is impaired and the attacks are successful. In studies on sugar maple, defoliation induced chemical changes in root tissues that were favorable for the growth of *A. mellea* (Parker and Houston 1971, Wargo 1972). Growth of the fungus was actually stimulated by extracts from defoliated trees (Wargo 1972).

Côté (1976) observed that stressed trees were more attractive to secondary organisms. Adults of *A. bilineatus* were attracted in greater numbers to trees that had been stressed by girdling. Côté also observed that trees that succumbed to borer attack had a proportionally greater decrease in growth rate after defoliation than similarly defoliated trees that survived. This rapid decrease in growth rate may have impaired the tree's ability to resist the borer attacks. Attacks were successful, and death of the tree followed.

In attempts to assign a major or minor role to secondary organisms associated with tree mortality after defoliation, controversy has arisen over their importance. Staley (1965) indicated that defoliation was the primary causal factor in decline and mortality

of scarlet oak, with *A. mellea* and *A. bilineatus* contributing to the problem along with other edaphic and climatic factors. Others have indicated that *A. bilineatus* plays a major role in mortality of oaks after defoliation (Nichols 1968, Dunbar and Stephens 1975), with *A. mellea* having a minor role (Dunbar and Stephens 1975).

Houston (1967, 1973, 1974) proposed in his concept of dieback and decline diseases, which includes decline and mortality after defoliation, that mortality results from a sequence of events that starts with stress, which predisposes trees to invasion by organisms that can attack and kill the trees. This concept was supported by work on mortality of oak after defoliation by the gypsy moth (Wargo 1977). Secondary organisms were involved in mortality. On some trees, *A. mellea* seemed more important; on others, it was *A. bilineatus*; on still others both organisms were equally severe. The results suggest that both organisms are involved and that trees die because photosynthesis, growth regulators, and water and nutrition relations are affected in the crown by the defoliator and the borer, in the main stem by the borer, and in the roots by the fungus (Wargo 1977).

Measuring Response of Trees to Defoliation Stress
Philip M. Wargo

Introduction

Whether the response of a tree to stress from defoliation is adverse or not will depend not only on how much and how often the foliage is consumed but also on the physiological condition—that is, tree vigor or health—at the time of defoliation (Graham 1963, Kozlowski 1969). Therefore, environmental factors that affect tree vigor can influence the effects of defoliation, including previous defoliation(s) (Wargo 1978a).

Tree vigor is simply the physiological condition of a tree at a particular time and is dependent on the physiological performance of that tree within a specific environment. A good indicator of tree vigor,

then, should reflect physiological condition both before and after defoliation and also indicate the magnitude of a tree's response to defoliation.

Crown dominance and condition have historically been used as expressions of a tree's physiological condition. Unfortunately, these measurements are not dynamic enough to be good indicators. Crown dominance is dynamic early in the growth of a stand of trees but becomes relatively static. Crown condition changes are more dynamic, but the rate of change is slow. For example, the effects of a single heavy defoliation may take up to 5 years to be expressed in crown condition changes (Campbell and Valentine 1972, Campbell and Sloan 1977). Because of this delay, crown conditon may not necessarily reflect the immediate physiological condition of a tree.

Every tree is unique and responds to the environment, stress included, in its own unique way. Thus for any set of environmental conditions there will be a range of tree physiological conditions. General vigor categories may not accurately reflect this range. Methods are needed that reflect the variability of physiological condition and that are dynamic enough to reflect immediate changes, in order to evaluate tree vigor prior to defoliation and the response of trees to defoliation. This will allow accurate predictions of the effects of defoliation—an important part of pest management decisions.

Within this framework, new indicators of tree vigor were evaluated. The criteria for selecting a potential indicator were that it normally reflect the tree's immediate physiological condition, not be subject to rapid and large fluctuation but be sensitive to stress, and be easy to measure.

Based on previous work with sugar maple and defoliation (Parker 1970, Parker and Houston 1971, Wargo 1971, Wargo 1972, Wargo et al. 1972) and work on electrical properties of stressed trees (Snow 1942, Parr 1943, Fensom 1955), starch content of the roots, stem biopotential, and resistance of the stem cambial tissues to electric current were evaluated as potential indicators of tree vigor and tree response to stress. The results of these studies are presented in the following sections.

Starch Technique

In work with sugar maple and defoliation (Parker and Houston 1971, Wargo 1971, 1972, Wargo et al. 1972) it became apparent that starch content of the roots might be a good indicator of tree vigor and tree response to defoliation. In trees from stands of sugar maple that had been defoliated by the saddled prominent, *Heterocampa guttivita* (Wlkr.), starch content of the roots was affected by a single defoliation. In the fall after a single heavy defoliation, starch content of these trees was significantly lower than in trees defoliated lightly or not defoliated. Further analysis showed that the frequency of defoliation was also reflected in the starch content of the roots (Wargo et al. 1972). Additional research indicated that starch was sensitive to the stress of defoliation, with changes apparent within 2 weeks after defoliation (Wargo 1972). These data and studies on the effects of drought (Parker 1970), air pollution (Miller et al. 1968), and defoliation of other species by other insects (Staley 1965) suggested that starch content of the roots was indeed sensitive to stress in general.

Studies were immediately established to evaluate the reliability of starch as an index of vigor and indicator of effects of stress, and also to determine the relationship of starch content and tree response to defoliation.

Measuring Starch Content of the Roots

The immediate problem was to find a method of measuring starch. To be useful to the land manager, the technique would have to be easy to use, simple to interpret, and require only little training and equipment to conduct. A major effort was placed on developing such a technique. Based on previous work by Parker and Houston (1971) and in an older report by Rather and Harrison (1939) a histochemical technique, Lugol's test (Johansen 1940), was tested and compared to a colorimetric technique after chemical extraction of starch (Wargo 1975a). The study indicated that with the histochemical technique roots could be sorted into categories that are probably

biologically important and could be used to indicate the effects of stress.

Further research showed that a single root sample was probably representative of the starch content of the tree (Wargo 1975*b*). Variation within roots was related to diameter and was attributed to the ratio of woody tissue to starch storage tissue. The study showed that the histochemical technique could be used to compare roots of the same tree or roots of different trees even though they had dissimilar diameters.

The proposed procedure for measuring starch content in the roots of trees is described in detail in Wargo 1978*b*. In brief, a sample from a large root is removed with a hammer and chisel and sectioned with a microtome. The cross section is stained with potassium iodide solution and rated as being either high, medium, low, or depleted in starch content. The starch is used as an indicator of the photosynthetic productivity, which is directly related to the well-being of a tree (Kramer and Kozlowski 1960). Trees with high starch content reflect high photosynthesis, and this is equated to high vigor. Low or depleted starch in a tree indicates that insufficient photosynthesis has occurred, which is equated with low or poor vigor, and that the tree has been stressed significantly.

The proposed technique was field tested and proved to be a practical test that land managers could use (Wargo 1978*b*). Over 50 samples were collected per day using two-person crews to collect the root samples. This crew also performed the starch test and completed 60 samples per day.

Starch Content and Decline of Defoliated Trees

At the same time studies were initiated to develop a technique to measure starch content, other studies were established to determine the relationship among starch content, defoliation, and tree decline.

An intensive study was conducted using artificial defoliation and small trees. The details of the study procedure are outlined in this section. The study was designed to determine the relationship of starch content of the roots at the time of defoliation to subsequent tree decline and mortality. The effects of each defoliaton on starch content and the relationship of the new starch content to effects of subsequent defoliations were also studied.

The starch content of the roots was analyzed both by chemical extraction and colorimetric quantification and by histochemical estimate (Wargo 1975*a*). In all cases there was an excellent relationship between the colorimetric estimate based on a percent of dry weight and the histochemical estimate (table 5-11). Trees determined to have high starch content based on chemical analysis were also rated as having high starch content based on the histochemical technique; trees with low starch content based on chemical analysis were rated as low starch trees. This supported previous work that indicated that, although not as

Table 5-11.—*The relationship of chemical determination of root starch content and histochemical estimates of starch content for defoliated (100 percent) and undefoliated black oak, white oak, and sugar maple trees, as indicated by coefficients of correlation*

| Species | Initial starch[1] | Autumn starch | | | | |
		1971	1972	1973	1974	1975
Black oak	0.787	0.851	0.929	0.887	0.855	0.881
White oak	.819	.865	.935	.923	.906	.941
Sugar maple	.887	.937	.934	.837	.896	.867

[1]Initial starch determined at the time of defoliation in May, June, July, or August.

precise as the colorimetric technique, the histochemical technique was accurate enough to be used as an estimator of starch content in both defoliated and undefoliated trees (Wargo 1975a).

There was a consistent association of low starch content and mortality in this study. Trees defoliated at the time of season when starch was low suffered greater mortality than trees defoliated when their starch was high. But even if trees were defoliated when their starch content was high, the trees with lower starch content suffered the greatest amount of damage after defoliation.

At the same time the intensive study was initiated on small trees, a field test of the starch-defoliation relationship was initiated in 1972. Ten plots each were established in three states: Massachusetts, New Jersey, and New York (see map in Reardon and Podgwaite 1976). Trees were selected near plots established in 1972 to monitor the gypsy moth intensively (Reardon and Podgewaite 1976, Campbell and Sloan 1978). A total of 25 trees were selected randomly from the red oak group (including *Quercus rubra* L., *Q. velutina* Lamarck, and *Q. coccinea* Muench.), from the white oak group (*Q. alba* L. only), or from both the red and white oak groups.

A single piece of root or wedge of root tissue was removed from each tree, dried, and ground in a Wiley mill (Wargo 1971). Starch content was determined by chemical extraction and colorimetric quantification according to the methods of Hassid and Neufeld (1964) and Siminovitch et al. (1953), respectively.

Root samples were taken for starch analysis in late winter/early spring in 1972, prior to any bud activity, and each autumn from 1972 through 1975. In addition, the percent of defoliation and percent of refoliation were estimated each growing season from 1972 through 1976. Mortality was recorded each year through autumn 1976.

Heaviest recorded defoliation occurred on all plots in 1972 and 1973 (table 5–12). Starch content in the red oaks was highest in spring 1972, decreased to a low point in autumn 1974, and showed signs of recovery as starch content increased in autumn 1975 (table 5–12). In all the plots in all the areas, the white oaks

had less starch than the red oaks did and, therefore, declined to lower values than the red oaks through autumn 1974. The starch content of white oaks had increased in autumn 1975 but did not recover to predefoliation levels even after 2 years of no or very low defoliation. This indicated that the effects of defoliation on starch are not expressed just during the years of defoliation.

Starch content data suggested that in all but the Cape Cod, Mass., area, heavy defoliation had occurred prior to 1972. Starch content in both oak groups in spring 1972 was much lower than would be expected for undefoliated trees. Starch content was very low in many of the white oaks and suggested that they had been stressed by defoliation more severely than the red oaks.

Total mortality was low, with only 25 trees out of 614 dying (4 percent) (table 5–12). Mortality in the white oaks was higher than in the red oaks. Only 7 of 384 red oaks died (2 percent), and 19 of 230 white oaks died (8 percent). Twelve of the 19 dead white oaks were on the Cape Cod plots.

Because of the limited mortality, statistical comparisons of starch content and mortality were not made. However, on 9 of the 12 plots where mortality occurred, the average initial starch content of the dead trees was substantially lower than the initial starch content of the surviving trees.

The second field test was established in 1975 and was designed primarily to evaluate the practicality of using the visual histochemical technique (Wargo 1975a) as an estimator of starch content. The test was also used to gather additional data on the relationship of defoliation, starch content, and mortality.

An area in the Bald Eagle State Forest in central Pennsylvania, heavily infested with the gypsy moth, was chosen for the study. The area had been reported to have had little defoliation in 1974, but heavy defoliation was anticipated in 1975.

Nine plots were established, each containing 25 red oaks (including red, black, and scarlet) and 25 white oaks (including some chestnut oak). Only codominant trees were selected along five transects that paralleled the slope. The transects were 2 chains apart.

Table 5-12.—*Change in starch content in the roots of red and white oaks after defoliation in 1972 and 1973 in six locations infested by the gypsy moth*

| Area and species | Number of trees | Average percent defoliation | | Percent starch | | | | | Total percent mortality |
| | | | | Spring | Autumn | | | | |
		1972	1973	1972	1972	1973	1974	1975	
Cape Cod, Mass.									
Red oak	79	85	60	19	8	6	4	10	1
White oak	43	99	85	12	4	2	1	3	23
Ludlow, Mass.									
Red oak	85	25	25	8	5	6	3	6	1
White oak	35	50	30	3	2	3	2	4	6
Whitehall, N.Y.									
Red oak	38	20	5	7	7	5	5	8	3
White oak	36	30	7	3	4	4	3	6	3
Cobleskill, N.Y.									
Red oak	31	25	2	4	5	4	—	6	0
White oak	34	25	2	2	4	3	—	6	0
Clinton, N.J.									
Red oak	99	20	65	8	6	4	6	7	2
White oak	76	30	50	6	4	2	4	5	5
New Lisbon, N.J.									
Red oak	55	90	95	12	6	3	5	6	2
White oak	12	99	100	5	4	3	4	6	0

The first five red oaks and white oaks along each transect were selected as study trees.

Each tree was assigned a tentative crown condition in March 1975 based on criteria of dead wood in the crown. This crown condition was later evaluated after bud break in 1975 and adjusted according to criteria previously used for judging crown condition (Wargo 1977a).

A single sample of wood was removed from a buttress root of each tree for starch content analysis (Wargo 1975a, 1975b, 1978c). Cross sections of the root piece were stained with iodine solution and rated as high, medium, low, or depleted in starch content. Trees were then assigned a risk rating based on starch content. High-starch trees were considered low-risk trees, medium-starch trees were considered moderate risk trees, and low- or depleted-starch trees were considered high-risk trees. The risk referred to the potential for dieback and mortality after additional defoliation in 1975 and subsequent years.

Trees were then observed periodically during each year from 1975 through autumn 1977 to determine percent defoliation, percent refoliation, crown condition changes, and mortality. Another root sample was taken in autumn 1977 to determine whether surviving trees had recovered based on root-starch content.

The initial starch analysis suggested that available information on defoliation history was probably in error. Most of the white oaks were low or depleted in starch and this indicated that most of the white oaks had probably suffered heavy defoliation in 1974 and possibly in 1973 (table 5-13). Some of the red oaks were also low in starch and may have suffered heavy defoliation prior to 1975. The probability that the white oaks had been defoliated heavily prior to 1975 is high, but this does not rule out other stress factors having affected the trees in this area. Regardless of the cause, the visual estimate of starch content indicated that many of these trees had experienced prior stress and were in poor physiological condition.

No trees died during 1975, but mortality after heavy defoliation in 1975 confirmed the judgment that many trees were in poor health (table 5-13). Out of 450 trees, 176 (39 percent) were dead by autumn 1977. Over half

Table 5-13.—*Cumulative mortality of trees in the red and white oak groups placed in three risk classes based on a visual estimate of starch content prior to heavy defoliation in 1975*

Species	Risk group[1]		Cumulative mortality				Percent of risk group that died
			1976		1977		
			Spring	Autumn	Spring	Autumn	
Red oak group	High	37	12	14	17	19	51
	Moderate	81	20	21	23	25	30
	Low	107	6	28	31	31	29
Total		225	38	63	71	75	33
White oak group	High	161	48	70	79	81	50
	Moderate	52	9	16	18	19	36
	Low	12	1	1	1	1	8
Total		225	58	87	98	101	45

[1]High risk = low or depleted starch, Moderate risk = medium starch, Low risk = high starch. Number indicates original number of living trees assigned that risk rating.

(96) of these trees died by spring 1976 and 85 percent by autumn 1976. The initial proportion of dead white oaks to dead red oaks was 60:40 and changed only slightly by 1977, to 57:43. A total of 33 percent of the red oaks and 45 percent of the white oaks died.

Mortality was highest among the poorer crown trees in both oak groups (table 5-14). However, it was higher than expected in good-crown, dominant trees after only one heavy defoliation (Campbell and Valentine 1972, Campbell and Sloan 1977). The majority of these good-crown trees (55 percent) that died were low or depleted in starch at the time of defoliation; this may indicate as previously suggested that these trees had been defoliated heavily more than once. The percent mortality of dominant, good oaks in these plots was similar to that for dominant and subdominant oaks defoliated for 2 years (Campbell and Sloan 1977).

There was a consistent relationship between risk rating and mortality after defoliation. In both oak groups the percentage of trees that died within each risk group was highest in the high-risk group, and the percentage was almost identical in each oak group (table 5-13). In the white oaks the lowest percent mortality occurred in the low-risk group. In the red oaks percent mortality in the low-risk group was

Table 5-14.—*Number of good- (1), fair- (2-3), and poor- (4-5) crowned trees that survived or died after 1 year of heavy defoliation in a mixed oak stand*

Oak group	Crown condition				
	1	2	3	4	5
Red					
Survived	90	55	3	1	0
Died	34	29	9	2	2
Percent dead	27	34	75	67	100
White					
Survived	70	49	6	0	—
Died	45	45	8	2	—
Percent dead	39	48	57	100	—

equal to that in the moderate-risk group and 3 times as high as in the low-risk white oaks. The bulk of these low-risk red oak trees died during late summer of 1976.

A regression analysis of starch content and mortality was performed using an average starch index for each species for each plot as the independent variable and various total mortalities as dependent variables. The average starch index was obtained by assigning numerical values to the visual rating: Depleted-1, low-2, medium-3, and high-4. Cumulative mortalities used for each species were spring 1976,

autumn 1976, spring 1977, and autumn 1977. Combined red and white oak mortalities by spring 1976, autumn 1976, and autumn 1977 were also used.

Negative coefficients of correlation occurred between species'root-starch content and their respective categories of mortality after defoliation (table 5-15). Plots with trees having low-starch indices had high mortality; those with high-starch indices had low mortality. Coefficients were consistently higher for white oak.

In general, starch contents of the surviving trees in autumn 1977 indicated that recovery from the effects of defoliation was occurring, but it was better in the red oak group (table 5-16). Among the red oaks, 137 of 150 surviving trees had medium- or high-starch ratings, while among the white oaks, 79 of 124 survivors had medium- or high-starch ratings. Most trees had starch contents in autumn 1977 that were

equal to or higher than their initial rating in starch content in 1975 (table 5-17). Only among red and white oaks rated high and white oaks rated medium were there significant numbers of trees lower than their original rating.

Combined results from the small tree study and the two field studies suggest that additional field testing of the starch technique should be done. Starch content does indicate whether a tree has been stressed or not, and there is a strong correlation between starch content and vulnerability to the effects of defoliation. The technique should be tested within a variety of sites and defoliation regimes to determine fully its value to forest managers. At present starch content can be used to indicate stress and, in conjunction with other general categories such as crown position and condition, as an aid in risk rating stands to make sound pest management decisions.

Table 5-15.—Relationship of starch index for each plot and species and cumulative tree mortality for each species, each combined species, and combined species as indicated by correlation coefficients.

Independent variable (starch)	Dependent variable (mortality)	Cumulative mortality			
		Spring 1976	Autumn 1976	Spring 1977	Autumn 1977
White oak	White oak	-0.887	-0.772	-0.826	-0.799
	Red oak	- .173	- .317	- .408	- .357
	Total	- .419	- .138	—	- .262
Red oak	White oak	+ .167	+ .138	+ .274	+ .285
	Red oak	- .419	- .138	- .279	- .262
	Total	+ .005	- .010	—	- .027

Table 5-16.—Starch ratings in autumn 1977 of surviving red and white oak trees heavily defoliated in 1975

Oak group	Starch rating				
	High	Medium	Low	Depleted	Total
Red	74	63	10	3	150
White	17	62	41	4	124

Source: Wargo 1975a.

Table 5-17.—*Comparison of final starch rating in autumn 1977 with initial starch rating in 1975 of surviving red and white oaks heavily defoliated in 1975*

Initial starch rating by species	Total surviving	Starch rating, autumn 1977			
		High	Medium	Low	Depleted
Red oak					
High	76	40	28	7	1
Medium	56	30	22	2	2
Low	16	4	11	1	0
Depleted	2	0	2	0	0
White oak					
High	11	1	8	2	0
Medium	33	9	14	10	0
Low	55	3	27	22	3
Depleted	25	4	13	7	1

Electrical Resistance Technique

Introduction

At the same time studies were initiated to evaulate root-starch content as an index of vigor, others were begun to explore the use of electrical properties of trees as indices of vigor. Electrophysiological measurements are essentially nondestructive and are easier and faster to measure than starch content.

Electrical properties of plants have been related to tissue vitality. Impedence measurements (associated with alternating current) of tissues has indicated temperature injury in conifers (Glerum 1962), frost hardiness in Douglas-fir (Van Den Driessche 1969) and alfalfa (Calder et al. 1966), proliferation disease in apple trees (Dostálek 1973), and discoloration and decay in living trees (Tattar and Saufley 1973). Biopotential has also been related to tree vigor. Snow (1942) and Parr (1943) related increased biopotential of forest trees to decreased vigor and susceptibility to insect attack. Later, Fensom (1955) observed elevated biopotentials in red pine, after they were sprayed with 2, 4–D herbicide.

Initial investigations of this study were conducted on biopotential and resistance to pulsed electric current rather than on impedence. Resistance to pulsed current overcomes some of the disadvantages of impedence (Fensom 1966) and least upsets the preexisting living conditions (Williams et al. 1964).

Electrical resistance (ER) was measured with a Shigometer, which is patterned after a device designed to detect discoloration and decay in living trees (Skutt et al. 1972). The meter was modified to measure minimum ER in the stem tissues by using uninsulated stainless steel needles and inserting them through the bark and just into the wood (Wargo and Skutt 1975).

Initial studies on biopotential and stress from defoliation indicated that deviations between defoliated and undefoliated trees were mainly the result of transpiration interruption and not the adverse effect of defoliation. Preliminary studies using the Shigometer to measure ER indicated that the ER was responsive to the effects of defoliation, and it was not directly related to transpiration changes. Biopotential studies were discontinued, and a small-scale field study was initiated to confirm the observations on defoliation and ER.

Electrical Resistance and Defoliation

ER measurements were made on four species of oak—red (*Quercus rubra*), black (*Q. velutina*), white (*Q. alba*), and chestnut (*Q. Prinus*)—in areas in Pennsylvania where heavy defoliation by the gypsy moth had occurred (Wargo and Skutt 1975). ER was affected by tree species, the red oak group (red and black) having a lower average ER than the white oak group (white and chestnut); by diameter, the larger trees having lower ER than the smaller trees; by crown

class, dominant trees having lower ER than subdominant trees; and by crown condition, good-crown trees having lower ER than poor-crown trees. However, regardless of the species, diameter, crown class, or crown condition, defoliated trees had on the average higher ER than similar undefoliated trees. This study established that trees having high-vigor characteristics had lower ER than trees with poor-vigor characteristics and that stress caused ER to increase.

The effects of defoliation on ER were further evaluated by making weekly or biweekly measurements on small, artificially defoliated trees. On trees that had been defoliated for 3 consecutive years prior to the ER measurements, ER, averaged for the growing season, was higher on defoliated trees in both growing seasons after defoliation (fig. 5–13). ER was

lower on the average during the second growing season and may have been an indication of recovery from the effects of defoliation; mainly, however, it reflected a higher average temperature for the second growing season.

ER measurements on these trees changed during the season, but the relationship of higher readings on defoliated trees was maintained throughout the growing season. ER was strongly influenced by temperature during both the early and later part of the growing season (fig. 5–14, *A, B,* and *C*). ER was inversely proportional to the temperature. However, there were changes in ER which were not entirely temperature dependent, especially in autumn. For example, ER readings were higher in the autumn than in the spring at similar temperatures, and ER in the
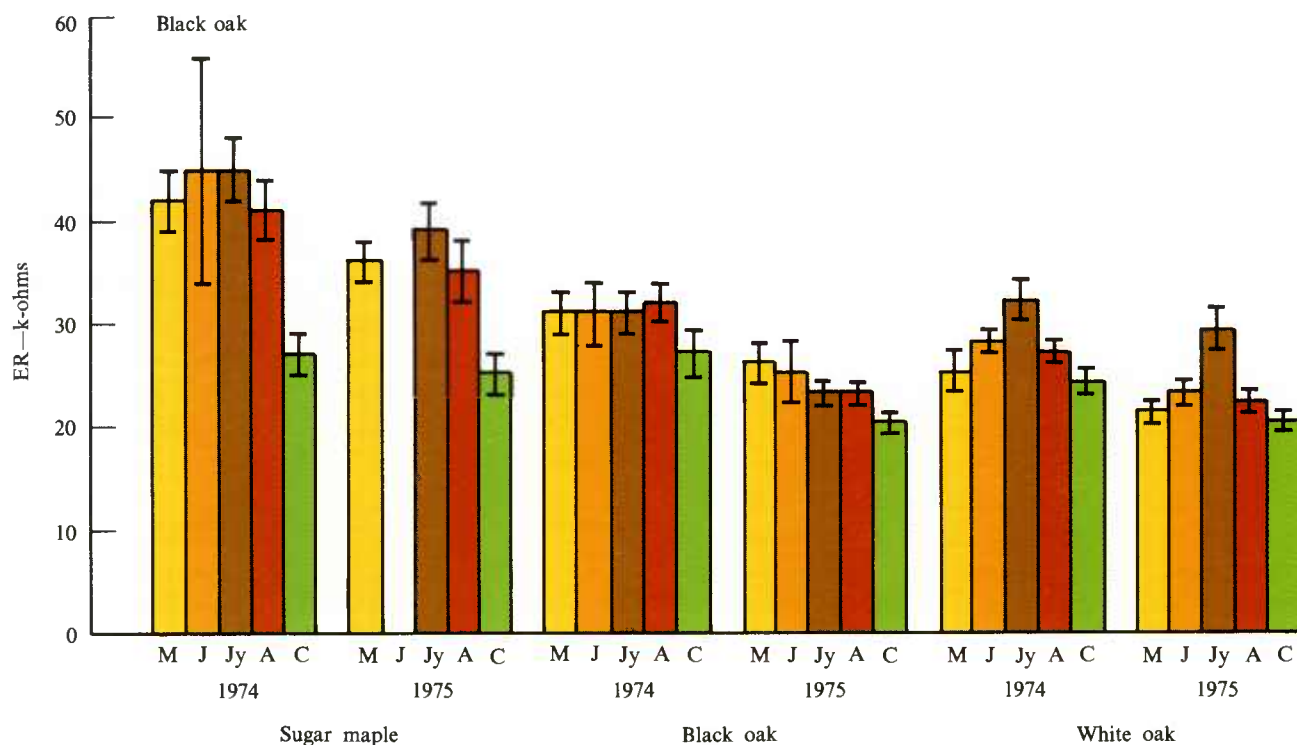
Figure 5–13.—*Average electrical resistance and 95 percent confidence limits for 1974 and 1975 for surviving sugar maple, black oak, and white oak that had been defoliated artificially (100 percent) in May, June, July, or August in 1971, 1972, and 1973. No June-defoliated sugar maple survived.*

spring was higher than at comparable temperatures later in the growing season. These measurements probably reflect changes in the tissues associated with the onset and termination of growth processes.

On other small trees, ER was measured prior to and after defoliation. Seasonal patterns were similar to those previously observed (fig. 5-15). Undefoliated and "to be" defoliated trees started at approximately similar ER's and remained similar until the defoliated trees began to refoliate. At that time, average ER on the undefoliated trees continued to decline, while in defoliated trees it increased. ER remained widely
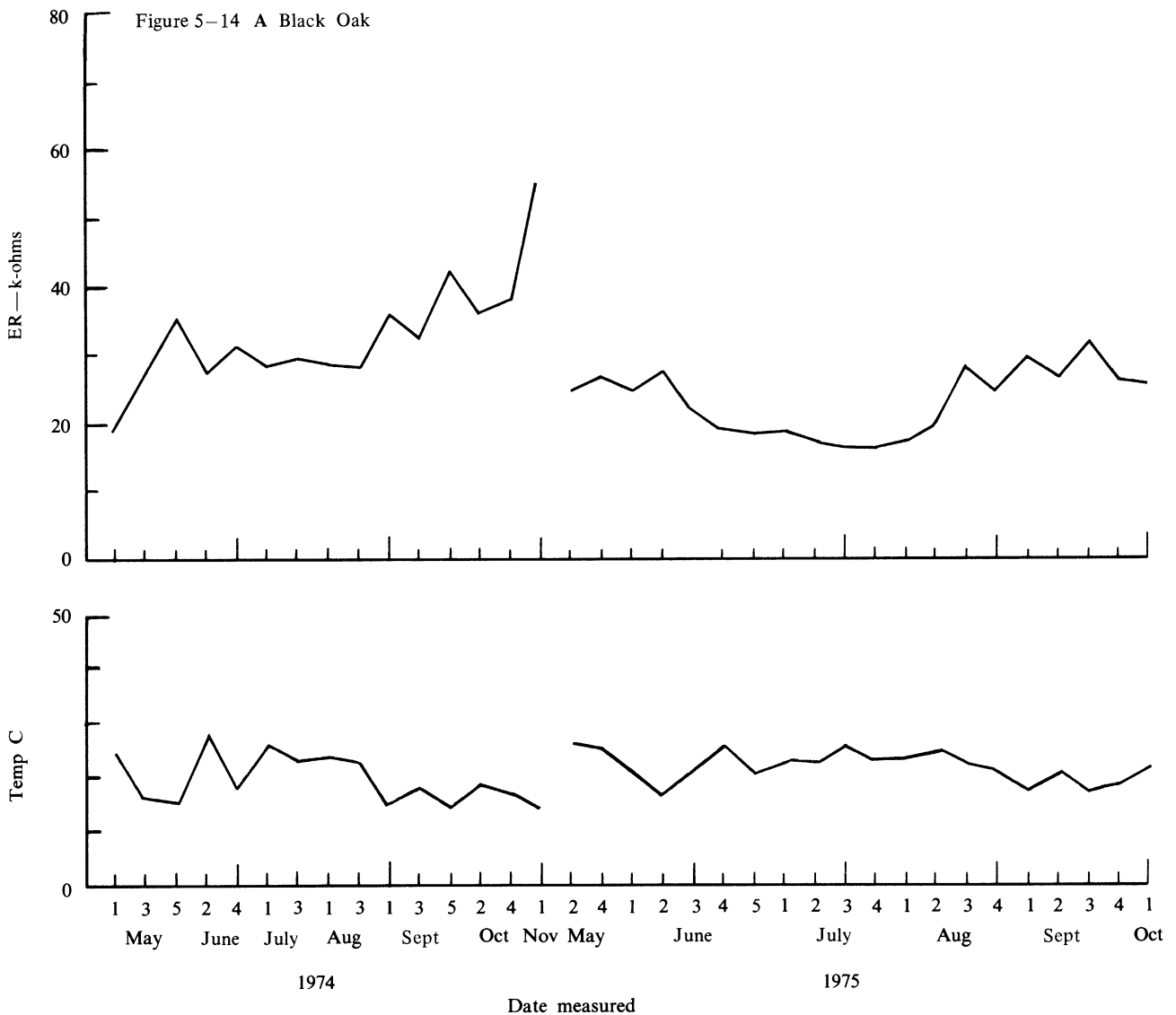
Figure 5-14.—*Seasonal changes in electrical resistance of stem tissue in trees and their corresponding temperatures for two growing seasons. ER measured biweekly in 1974 and weekly in 1975:* A, *Black oak;* B, *white oak;* C, *sugar maple.*

Figure 5–14 **B** White oak
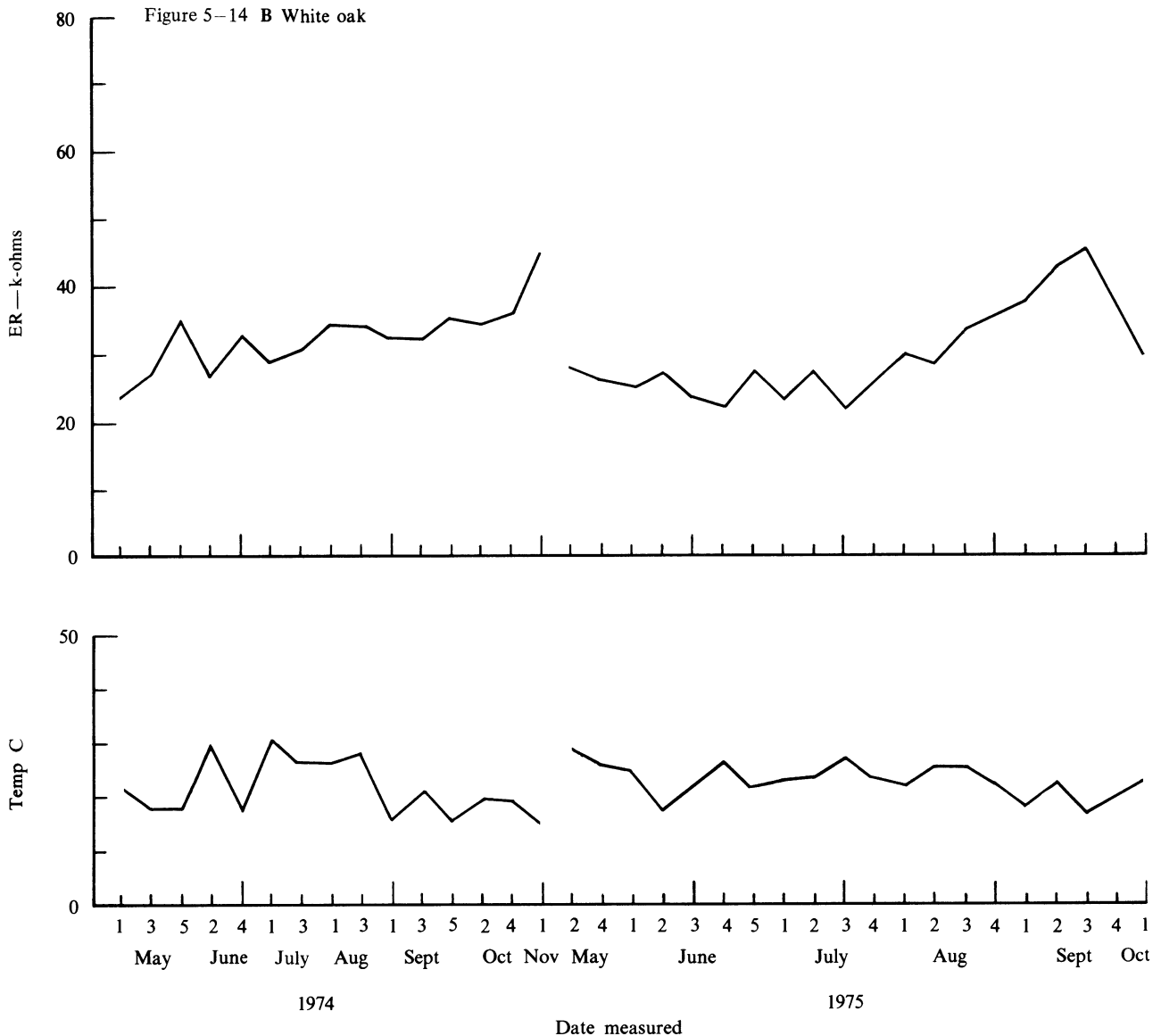
separated into October, but then differences became less through mid-November. This study confirmed the hypothesis that defoliation caused ER to rise and that differences observed in the initial study when ER was measured after defoliation were the result of defoliation, not inherent differences prior to defoliation.

In addition to the relationship of high ER and stress by defoliation, another study on small trees showed a relationship between ER and wound closure in defoliated and undefoliated sugar maples (Wargo 1977*b*). Trees were wounded in spring 1974, after 3 years of complete defoliation. Wound size was measured in autumn 1975 after two complete growing seasons, and ER was measured during both growing seasons. In both defoliated and undefoliated trees, wound closure was significantly and positively correlated with ER. Undefoliated trees had lower ER

Figure 5–14 C Sugar maple
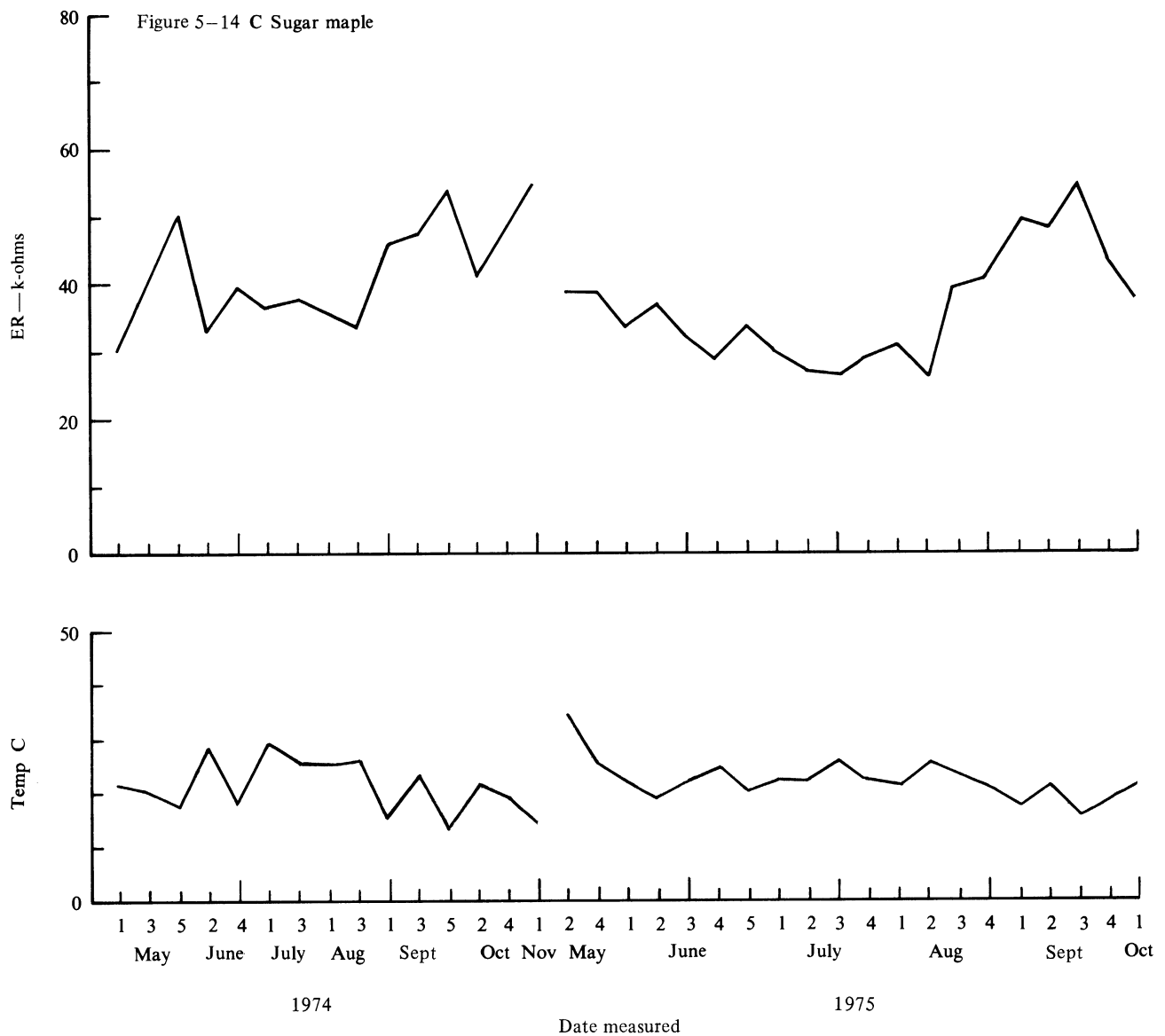
readings and had smaller wound openings than defoliated trees, indicating less adverse response to the wounding and a more rapid rate of closure. Among the defoliated trees, trees with lower ER readings also had proportionally smaller wound openings. There was also a significant correlation between ER and starch content; ER in trees with low starch content was much higher than in trees high in starch. This relationship was also observed in oak trees defoliated by the gypsy moth (Côté 1976), indicating that both ER and starch content reflect the effects of stress on trees.

Factors Influencing Electrical Resistance

Results of the initial study relating high ER and defoliation by the gypsy moth (Wargo and Skutt 1975) indicated that diameter significantly influenced
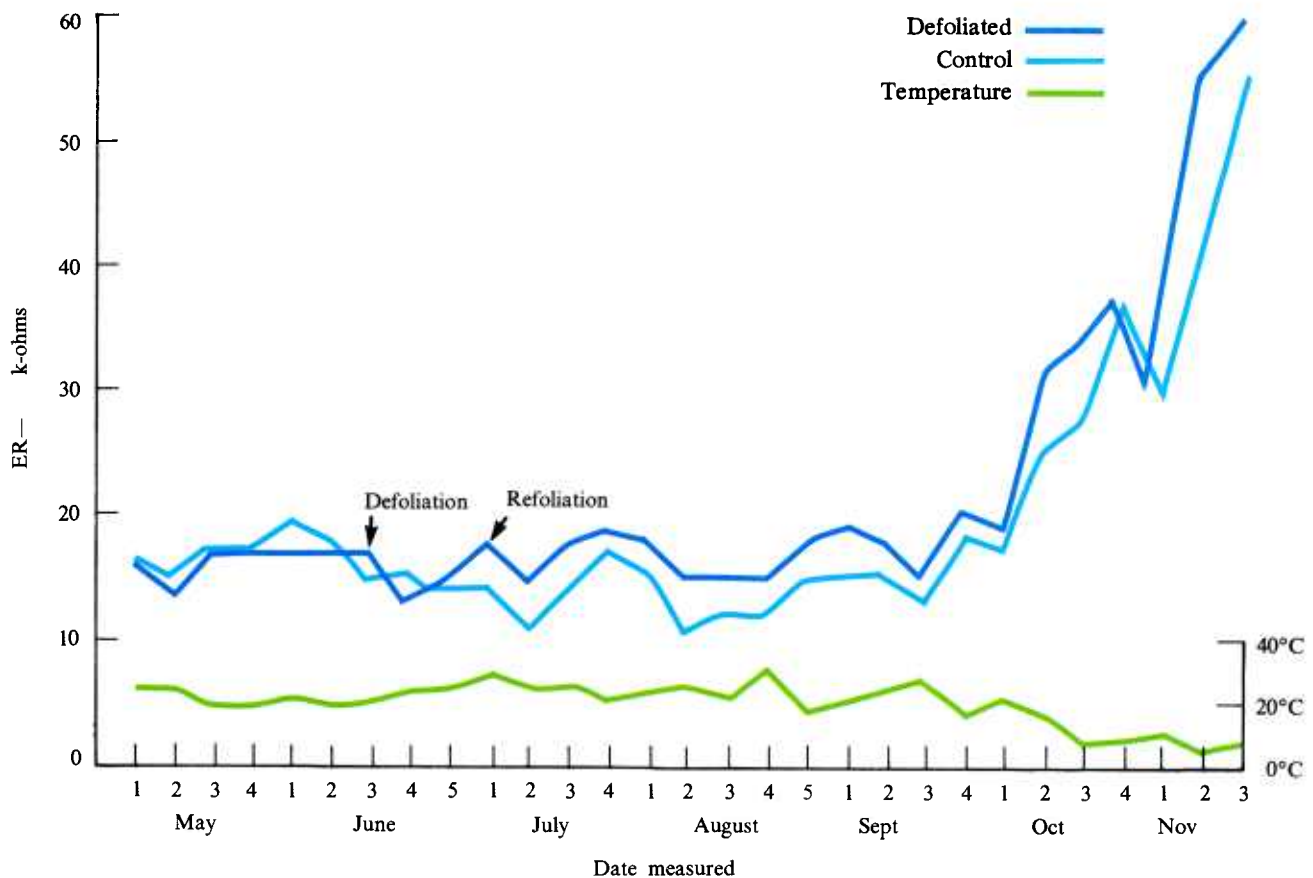
Figure 5-15.—*Seasonal changes in electrical resistance of black oak trees in response to defoliation and temperature changes.*

the ER measurements. There was a negative correlation between diameter and ER. To determine whether physical diameter was influencing ER, or whether diameter simply expressed the tree's physiological performance as reflected in ER, a series of ER measurements was made at different heights and consequently at different diameters on the same tree. Twenty-five red oaks and 25 white oaks averaging 22 cm and ranging from 15 to 34 cm diameter at 1.4 m were used. ER readings were taken every meter from 0.3 to 5.3 m above the soil. At each point the diameter was measured and an increment core was removed. The width of the sapwood, the number of growth rings in the sapwood, and the width of each ring for the last 5 years were measured.

With increasing height, tree diameter decreased uniformly in all trees (table 5-18). However, ER did not increase correspondingly. In red oak, there was a trend for ER to decrease with increasing height, but it was not significant. In white oak, ER was lowest at 0.3 m, increased at 1.3 m, and then progressively decreased with increasing height. The average change in diameter was approximately 11.5 cm and the corresponding change in ER was less than 1 k ohms. No linear relationship existed between ER and physical tree diameter.

The average ER readings were higher for white oak than for red oak, but average diameter was higher in the white oaks. Sapwood width was uniformly greater in the white oaks, but the number of rings in the

Table 5-18.—*Relationship of average diameter at different heights above ground, average sapwood width, and average number of rings in the sapwood, with average ER in red and white oak trees*

Variable, by species	Height above ground (m)					
	0.3	1.3	2.3	3.3	4.3	5.3
Red oak						
Diameter (cm)	30.0	22.5	21.5	20.2	20.0	18.7
Sapwood width (mm)	14.1	12.1	11.2	11.3	10.9	12
Rings in sapwood	11	10	10	10	10	10
ER (K-ohms)	8.8	8.7	8.3	8.8	8.3	8.1
White oak						
Diameter (cm)	31.0	22.9	21.7	21.2	20.2	19.1
Sapwood width (mm)	22.2	16.7	15.6	16.5	16.2	16.4
Rings in sapwood	18	16	16	16	16	17
ER (ohms)	9.2	10.5	10.3	10.0	9.9	9.8

Table 5-19.—*Multiple R^2 values from stepwise regression analysis of ER and the proportion of the value accounted for by diameter and sapwood width*

Variable, by species	Height above ground (m)						
	0.3	1.3	2.3	3.3	4.3	5.3	Tree[1]
Red oak							
Multiple[2]	0.85(3)	0.37(1)	0.75(4)	0.71(3)	0.45(1)	0.59(1)	0.73(2)
Diameter	.17	0	0	0	0	0	0
Sapwood	.54	0	.48	.53	.45	.59	.67
White oak							
Multiple[2]	.65(3)	.64(2)	.71(3)	.62(2)	.42(2)	.88(4)	.66(2)
Diameter	0	0	.54	.46	0	0	0
Sapwood	.37	.48	.10	.16	.30	.50	.49

[1]Data averaged over all heights.
[2]Number in parentheses indicates number of independent variables.

sapwood was also greater in the white oaks. This resulted in a slightly greater average ring width in the red oaks of 1.14 versus 1.0 in the white oaks, which may account for the lower ER readings in red oaks in spite of the smaller diameter.

Height data were analyzed using a multiplelinear-stepwise regression program to indicate the most important variables affecting ER. Total sapwood width (in contrast to heartwood) and not diameter of the tree (trunk) emerged as the single most important independent variable (table 5-19). In white oaks it occurred as an important variable in all regression analyses for each height and height data averaged for

each tree. In the red oaks it appeared as the most important variable in the height averaged regression and in all but one height regression. Diameter was an important variable in white oak only at 2.3 and 3.3 m. In red oak, diameter appeared as an important variable only once, at 0.3 m.

Results indicated that although there was correlation between ER and diameter, it was not physical size that was influencing the readings. Rather, ER was reflecting a property of the tree that influenced growth rate and hence diameter. However, average growth rate over or in any of the last 5 years was not consistently indicated as an

important variable. These results corroborate a report by Shortle et al. (1977) that physical diameter did not influence ER.

Reevaluation of Techniques to Measure Electrical Resistance

Attempts to correlate ER and decline and mortality of trees have, thus far, been inconsistent even in obvious decline situations (Newbanks and Tattar 1977). Analysis of the techniques used for determining minimum resistance indicates that perhaps altered ER in the cambial area has not been detected or measured. When uninsulated probes are inserted through the bark into the wood, the tree tissues act as resistors in parallel (Carter and Blanchard 1978). Resistance indicated is not the resistance of the tissue with the lowest resistance but total minimum resistance that is that tissue's resistance reduced by whatever current passes through the adjacent tissues. Thus for two adjacent tissues, similar in resistance, a change in the resistance of one tissue may not significantly influence the overall resistance reading, especially if the area of that tissue is small.

Resistance is also dependent on probe-tissue surface area contact. The cambial zone, which is probably most sensitive to stress, is relatively small compared to the inner bark and wood cylinder, and there is less probe-cambial surface area contact than in xylem and phloem. Changes in ER could be occurring in response to stress in the cambial zone but are masked because the resistance in the adjacent and larger tissues is similar to or lower than that in the cambial zone and there is greater probe-surface area contact.

The original reason for using uninsulated probes was to avoid the need to determine the exact depth to which probes had to be inserted to measure minimum resistance, or to make sequential (depth) readings to find minimum resistance. It seems that the uninsulated probes, although circumventing the depth problem, may create another.

To determine the difference in ER measured with insulated and uninsulated electrodes, ER measurements were made at different depths on black oaks, white oaks, and sugar maples. Measurements were made with probes of similar diameter that were insulated up to the tip or uninsulated their whole length (fig. 5-16). The probes were spaced 5 cm apart and were inserted gradually into the tree to a depth of 20 mm, and ER was recorded every 2 mm. When ER was measured with the uninsulated probes, there was a progressive decrease in ER and then no change after approximately 10 mm. When ER was measured with the insulated probes, it decreased, reached a minimum, and then began to increase. (fig. 5-17, A, B, and C). Minimum resistance measured with the insulated probes was always higher than that measured with uninsulated probes. The depth for minimum ER measured with insulated probes corresponded to approximate bark thickness. This indicated that the minimum resistance was occurring near the cambium. There was also some indication that measurements with the insulated probes were less variable per tree than measurements with uninsulated probes.
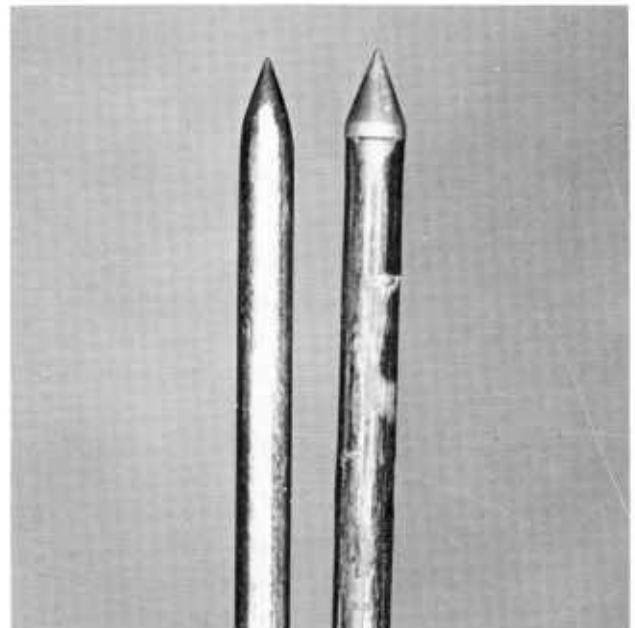
Figure 5-16. —*Stainless steel electrode uninsulated the whole length (left electrode and uninsulated just at the tip (right electrode).*

To gain additional information on the variation of ER measurements using insulated and uninsulated probes, measurements at each of the cardinal directions were made on large trees, 15 to 40 cm in diameter at 1.4 m in both the red (148) and white (129) oak groups on nine different plots in oak forests in central Pennsylvania. The uninsulated probes were the standard 28 mm stainless steel electrodes (fig. 5-18) used in previous studies (Wargo and Skutt 1975). The insulated probes were from Delmhorst Electronics, Boonton, N.J., and were larger in length and diameter that the uninsulated probes (fig. 5-18). Because of their larger diameter, ER readings were expected to be lower than those made with uninsulated probes because of increased probe-tissue surface area contact. The variation differences would still be expressed if they did indeed exist.

Because the insulated probes increased rapidly in diameter to form a collar that separated the insulated portion from the uninsulated portion, they were blunter than the uninsulated probes (fig. 5-19). This feature allowed the insulated probes to penetrate easily through the bark but not through the wood unless considerable force was applied. This resulted in the probes penetrating to but not through the wood and put the uninsulated portion of the tip in the cambial area, and therefore in the tissue, with the minimum resistance.

The mean and standard deviations of the ER measurements were calculated for the four insulated
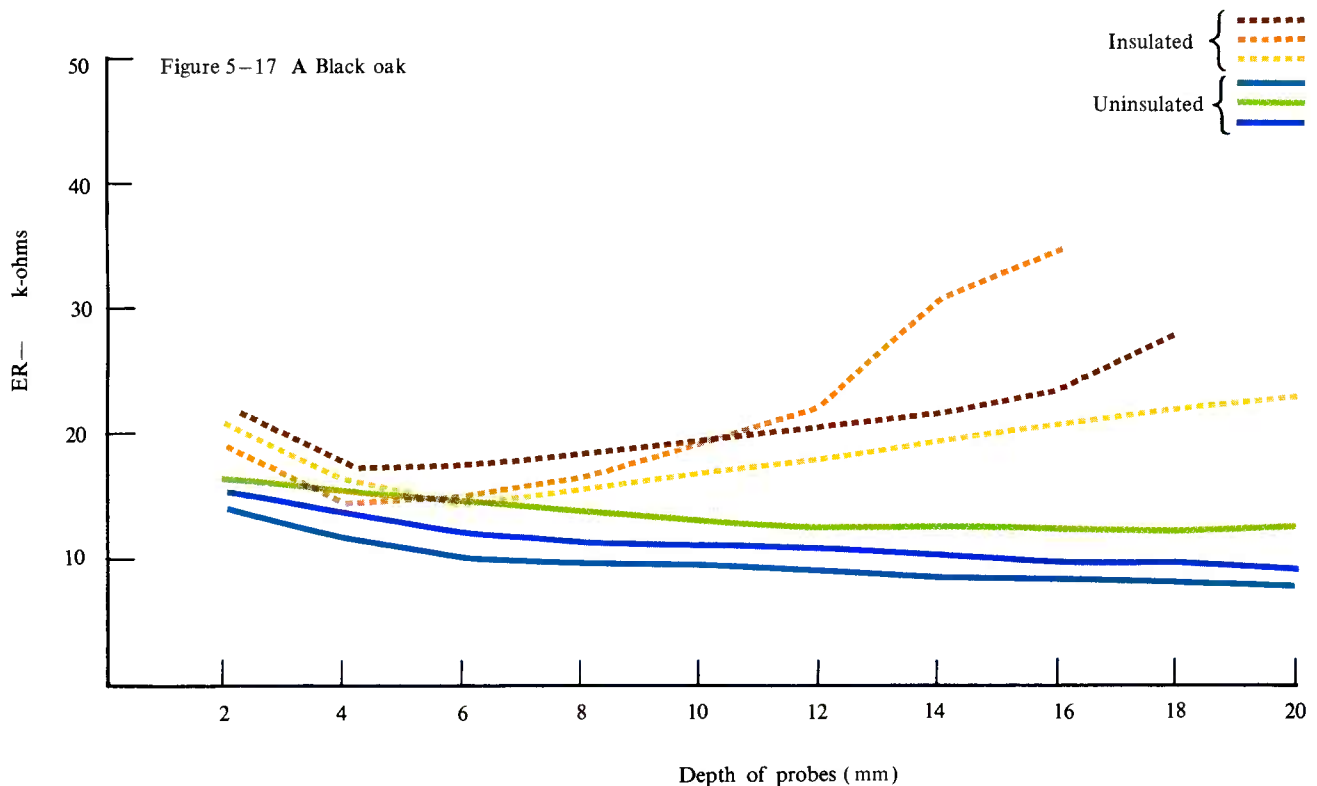
Figure 5-17.—Comparison of the pattern of electrical resistance change with depth of electrode measured with insulated and uninsulated electrodes inserted through the bark and into the wood in 2 mm increments: A, Black oak; B, white oak; C, sugar maple. Three trees of each species were measured.

Figure 5–17 **B** White Oak

Insulated

Uninsulated

ER— k-ohms

Depth of probes (mm)

Figure 5–17 **C** Sugar Maple

Insulated

Uninsulated

ER— k-ohms

Depth of probes (mm)
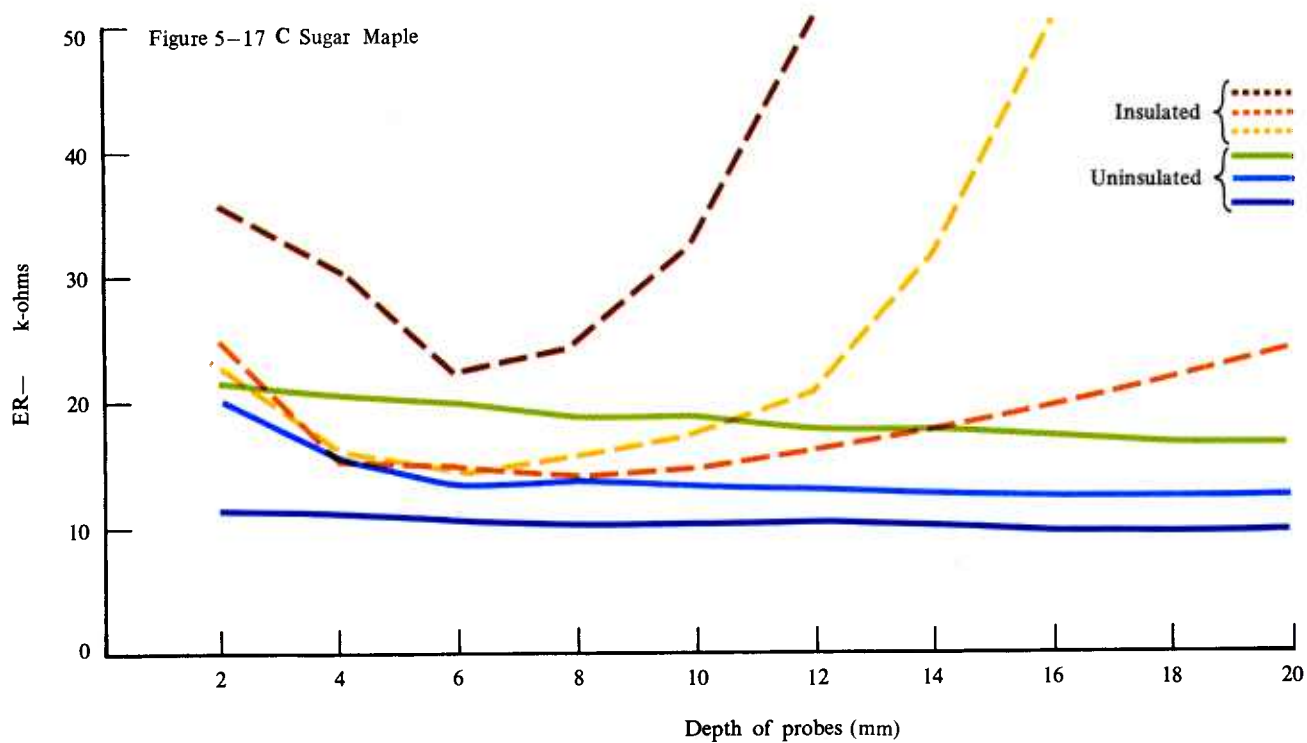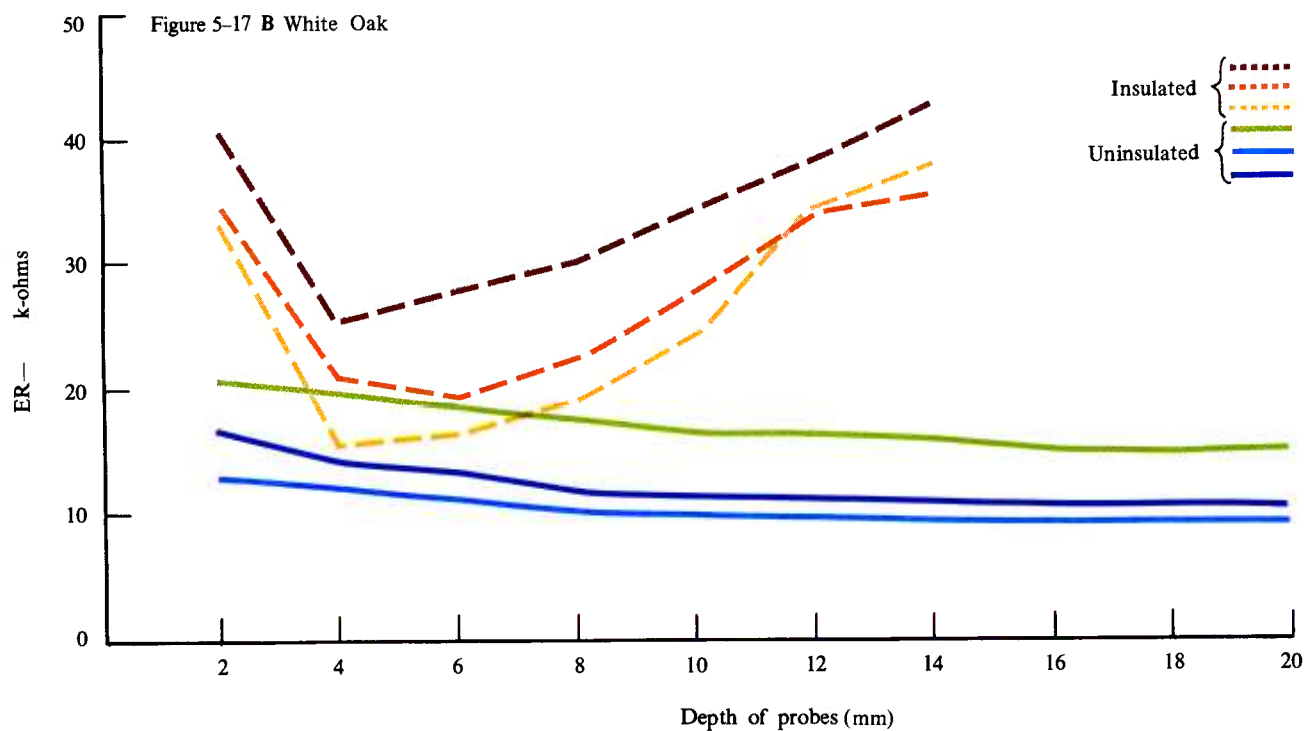
and four uninsulated readings on each tree. Since there was a consistent difference in the means because of probe size and a trend to higher standard deviation with the higher means, coefficients of variation were determined by dividing the standard deviation by the mean and expressing it as a percent of the mean.

The coefficient of variation in the majority of comparisons was higher for the uninsulated probes in both the red and white oak groups (table 5–20). In the red oaks, the ER measurements with uninsulated probes had a 50 percent higher coefficient of variation on 38 percent of the trees, while the ER measurements with the insulated probes had a 50 percent higher coefficient on 19 percent. In the white oaks, ER measurements with uninsulated probes had a 50 percent higher coefficient on 43 percent of the trees, and ER measurements with the insulated probes had the

higher coefficient on 18 percent of the trees. When separated into their respective plots, the average coefficient of variation was higher for ER measurements with uninsulated probes in all but one plot each of the red and white oak groups (table 5–21).

Carter and Blanchard (1978) found that ER measurements were significantly and highly negatively correlated with phloem thickness in red maple. Since phloem thickness varies from tree and also within a tree, it could be responsible for considerable variation in the electrical resistance measurements. The insulated probes bypass this phloem influence and hence eliminate the variation imparted by it. These data indicate that measurements with the insulated probes, if they are indeed better measures of ER and stress-related phenomenon, would also be, because of reduced variability, superior measurements for separating trees into various stress groups based on ER.
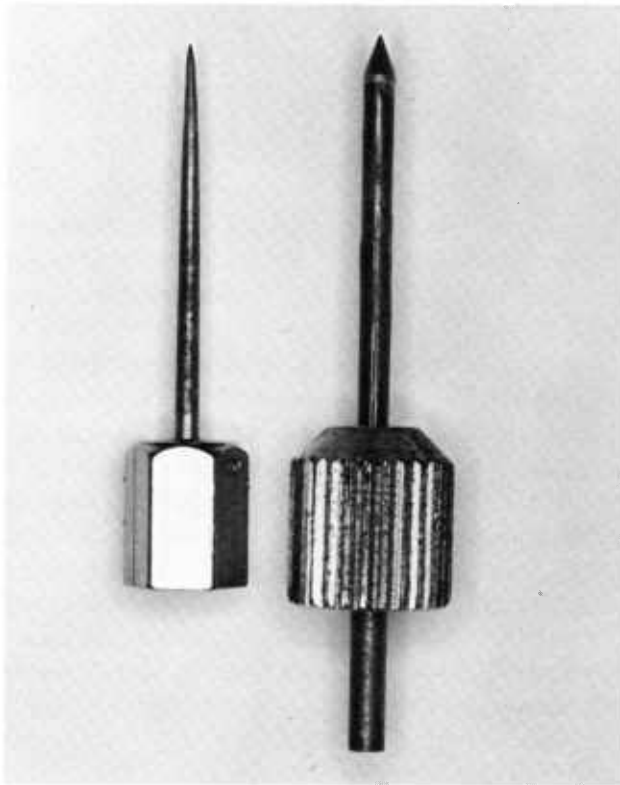
Figure 5-18. —*Standard uninsulated (left) and insulated (right) stainless steel electrodes used for the variation analysis.*
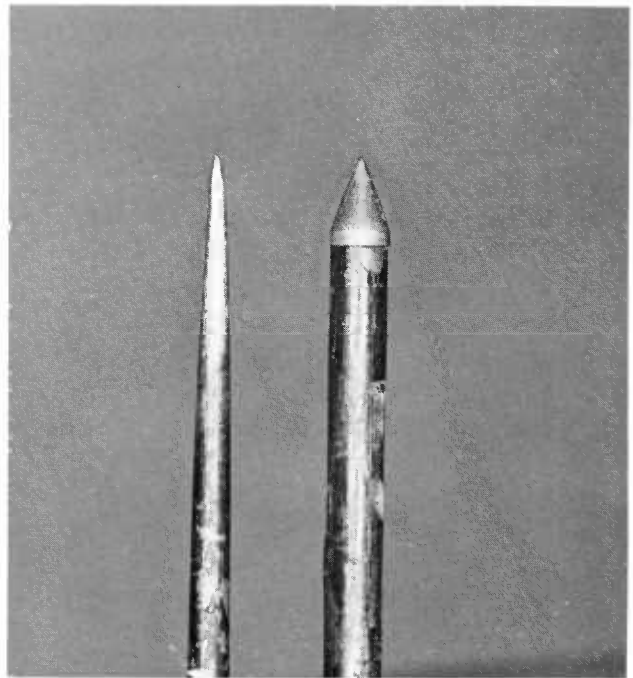
Figure 5-19.—*Close up of the tips of the standard uninsulated and the insulated showing the blunter and larger tip and the slightly raised collar to protect the insulation.*

Table 5-20.—*Number of comparisons where coefficient of variation for ER measurement using uninsulated probes was 50 percent greater than the corresponding coefficient of variation for ER measurements using insulated probes*

Oak group	Uninsulated greater	Insulated greater	Neither greater	Total
Red oak	55	28	65	148
White oak	55	23	51	129

Table 5-21.—*Average coefficient of variation for four uninsulated and insulated probe ER measurements for red and white groups on nine different plots*

	Coefficient of variation					
	Red oak group			White oak group		
Plot number	Number of trees	Uninsulated	Insulated	Number of trees	Uninsulated	Insulated
1	16	0.126	0.086	10	0.082	0.070
¹2	¹22	.096	.098	21	.102	.095
3	16	.154	.085	13	.106	.067
4	21	.101	.086	13	.094	.075
5	18	.111	.098	9	.132	.053
6	20	.111	.095	15	.087	.076
7	14	.097	.082	15	.083	.066
¹8	12	.111	.086	¹15	.106	.104
9	9	.136	.116	18	.092	.082

¹Denotes plot and species where coefficient of variation was not higher for the uninsulated probes.

Conclusion

Results are far from definitive in the use of electrical resistance as an index of vigor. However, these results, plus the results of others working with electrophysiological techniques, indicate that ER has the potential to be used as an index of vigor. Shortle et al. (1977) found a relationship between ER and sprout vigor in clumps of red maple; regardless of diameter, the largest member of each clump had the lowest ER. They also related low ER to rapid wound closure in hybrid poplar (Shortle et al. 1977). ER has also been used to show the effects of fertilization in white birch (Shigo et al. 1975, Smith et al. 1975) and symptoms of vascular wilt disease in elm (Carter and Blanchard 1976) and sugar maple (Malia and Tattar 1975).

An example of the problems encountered using ER to indicate vigor was shown by Newbanks and Tattar (1977). They found no relationship between declining crown conditions of urban maples and ER. There was also a poor relationship between ER and trees stressed by drought, root pruning, and soil compaction. However, in nonurban sugar maple trees good crowned trees had lower ER readings than declining trees.

Perhaps some of the difficulties encountered in relating ER to various indicators of stress to vigor is related to the technique of measuring ER with the uninsulated probes. Measurements with these probes can be influenced by many tree properties, including inner bark thickness, growth increment, sapwood thickness, and in some cases depth of the probes, and probably many other properties of the individual tissues. The influence of this many factors can create large variations in readings; hence it is difficult to standardize measurements and interpret ER measurements in response or relation to a particular tree property.

The technique of ER measurement needs to be explored fully to determine in what tissue ER measurements are most meaningful and to develop technique(s) to measure it. Until that is accomplished

ER can be used only as a general aid in making decisions about trees.

Forest Stand Relationships

David R. Houston

Characterization of Susceptible and Resistant Forests

Gypsy moth defoliation records for the New England States and New York reveal that some oak forests have a history of repeated defoliation, while other stands have been defoliated rarely. These records also show that outbreaks often begin and last longer in the susceptible, often defoliated forests, but in the rarely defoliated resistant stands, the outbreaks were short lived and occurred only when large numbers of insects were blown in from nearby areas.

Bess et. al. (1947) characterized these New England forests on the basis of species compostion and history of disturbance. In susceptible stands, the dominant trees—usually aspen (*Populus tremuloides*), gray birch (*Betula populifolia*), scarlet oak (*Quercus coccinea*), white oak (*Q. alba*) and black oak (*Q. velutina*)—are short and scrubby their crown canopies often covering less than half the ground. Ground-cover species common in these areas include lowbush blueberry (*Vaccinium* spp.), sweetfern (*Comptonia peregrina*), bracken (*Pteridium latiusculum*) and, when sufficiently open, grasses and sedges. Litter layers are often thin or lacking in susceptible sites. Susceptible stands characteristically grow on adverse sites such as the excessively drained sands of the Atlantic coastal plain and dry, rocky ridges.

In contrast, resistant stands in New England commonly grow on deep loamy soils and possess well-developed litter layers. Resistant stands are well stocked with vigorously growing trees. Species typical of these stands include red oak (*Q. rubra*), some white and black oaks, sugar maple (*Acer saccharum*), white ash (*Fraxinus americana*), black birch (*B. lenta*), yellow birch (*B. alleghaniensis*), and occasionally hemlock (*Tsuga canadensis*). When abundant, certain understory plants are characteristic of mesic forests and include such species as wild sarsaparilla (*Aralia nudicaulis*), maple-leafed viburnum (*Viburnum acerifolium*), and several woodland ferns (*Thelypteris noveboracensis* (*Dryopteris noveboracensis*), *Dryopteris spinulosa*, and *Polystichum acrostichoides*).

Bess et al. (1947) affirmed the fact that species highly favored by the gypsy moth occur in susceptible stands—but also pointed out that preferred species occur in resistant stands, often in abundance. Indeed, the main thrust of this classic work was to emphasize that it is not the presence or even the amount of preferred food species that renders a stand susceptible. Rather, it is the underlying ecological conditions—often resulting from disturbance—that create a favorable environment for the insect.

From the brief descriptions just given, it is clear that susceptible forests usually are on poor sites, rendered so by steep and exposed physiography or thin or excessively drained soils, or by disturbance, natural or manmade. The association of disturbance and susceptibility involves a number of possible relationships. By injuring and disfiguring trees, windstorms, ice and snow, fires, and heavy logging can create resting places (spatial niches) for gypsy moths. Furthermore, these agents, by opening up a stand, can dry out the forest floor and reduce the litter layers where small mammals and insect predators of gypsy moths normally abound.

Trees able to survive and grow on sites impoverished in moisture (xeric) and nutrients often reflect this poor site quality by growing slowly, remaining small, and developing features of structure or form that offer shelter for one or more stages of the gypsy moth. Such sites support forests whose successional progress is arrested by edaphic factors or disturbance. Experience over the past century has clearly demonstrated that the gypsy moth both affects and is affected by forest succession.

Forest succession, defined as the relatively orderly biological development of the forest, is a complex process. In mesic (moderately moist) northern New England, the development of hardwood forests, following agriculture or disturbance by heavy logging or fire,

often begins with stands rich in such short-lived pioneers as gray or white birch and aspen. Later, these species are replaced, first with early-stage, longer lived oaks and eventually by more shade-tolerant species such as red oak, sugar maple, beech, and hemlock. Most oaks in these forests are relatively transient, and their dominance of the site is usually for short periods of time. Such forests are typical of those classed as resistant by Bess et al. (1947).

Moderately heavy defoliation of these forests by gypsy moths often serves to accomplish, in a short time, what succession would do eventually, especially if forest development is well advanced (Bess et al. 1947, Campbell and Sloan 1977, Stephens and Hill 1971). This is because defoliation tends to reduce the relative proportion of highly preferred early-stage species, leaving forests richer in nonpreferred species. Severe, repeated defoliation, however, can result in considerable tree mortality, even among the generally less favored species. Stands opened up sufficiently to dry out soil and litter can be set back successionally.

In southern New England, oaks tend to be more numerous and to dominate the forest for longer periods. But here, too, records taken over time indicate that, barring disturbance, the importance of oak has continually diminished for at least the past 40 years (Stephens and Waggoner 1970).

Within the New England area, however, certain sites support forests whose development is regularly arrested by disturbance or by soil moisture deficits. Dry, rocky ridges and excessively drained sandy sites often support oak climax forests These sites, typically classed as susceptible by Bess et al. (1947), often suffer from prolonged severe defoliation. Tree mortality can be high if defoliation is sufficiently intense, especially if it occurs during unusually dry periods.

Heavy tree mortality can drastically set back forest development to early pioneer stages. Some cases have been reported where most of the trees were eliminated from the stand. A major concern to land managers deals with the fate of forests to the west and south of the old outbreak areas. It is apparent that the coastal plains of New Jersey and States further south support oak climax forests as does an increasing acreage of Appalachian ridge tops in the mid-Atlantic region. It is likely that in these latter stands, forest development can only be adversely affected. The coastal sand plains oak stands, however, have often evolved from earlier pine forests. Here oak mortality could actually be a boon to a forest management geared toward pine production.

Models to Classify Forests as Susceptible or Resistant to Gypsy Moth

The preceding points out that classifying forest susceptibility is a highly subjective process that requires an intuitive integrating of such diverse factors as overstory and understory vegetation, stand structure and history of disturbance, soils, and site physiography. All of this must then be filtered through layers of personal experience with the nature and whereabouts of past insect outbreaks.

The study objective was to develop a less subjective way to classify and perhaps even to predict a forest's susceptiblity. Because classification of forests outside the gypsy moth infestation area was desired, the method could not incorporate measurements of the insect or of its population trends but had to be based on the forest itself. The approach was to quantify some of the features shown by earlier workers to be characteristics of susceptible and resistant forests. Specifically, forests were classified on the basis of their species composition and with respect to the food and shelter they offer the gypsy moth.

Details of the methods used have been published (Houston 1975, Houston and Valentine 1977). Briefly, in addition to the species, size, and number of trees and saplings, six tree-structure features were also measured, all of which offer favored sites for resting, pupation, or oviposition by the insect, and most of which reflect either the tree's response to disturbance or are inherently characteristic of trees growing on poor sites. These features include the numbers of bark flaps, holes and wounds, large lower dead branches, and dead sprout stubs and the presence of crook and of deep bark fissures.

The study began in 1972 and 1973 in New England, New York, New Jersey, and Pennsylvania with the sampling of stands, many of which had a history of

gypsy moth defoliation (fig. 5-20). Also sampled-were 13 stands in West Virginia where no gypsy moth outbreaks had ever occurred. Included were stands that had been defoliated often in the past (called susceptible), stands that had rarely been defoliated in the past (called resistant), and some stands that were being defoliated by gypsy moth for the first time.

To analyze the data two ordination techniques were used to compare stands on the basis of their species composition or their structural features. Sometimes the structural features were first ranked by the gypsy moth food-preference class of the tree species on which they occurred. The Bray-Curtis (B-C) ordination method (Bray and Curtis 1957, Beals 1960) and a principal-components analysis (PCA) ordination method (Seal 1964) were used. These multivariate techniques have received wide attention in the past several years as methods for analyzing complex community relationships (Curtis 1959, Gittens 1965, Allen and Skagen 1973, Levandowsky 1972). In the B-C analysis, stands were compared by the similarity index $C = 2w/a + b$. In this index, w is the sum of the lesser of the two scores for each feature, a is the sum of the

Figure 5-20.— *Areas where forests were sampled in 1972 and 1973 (118 stands) and in 1974 (50 stands).*

percent of the maximum scores for all features for one stand, and b is the same for each of the other stands.

Comparison of Stands on the Basis of Their Species Composition

Species composition has been considered an important determinant of susceptibility. A PCA ordination was performed to compare the 118 stands on the basis of their species' importance values (IV's), which consist of the sum of three components: The percentage of the plots in each stand containing the species, the species' percentage of the total stems, and the species' percentage of the total basal area.

Stand positions in the PCA IV ordination are identified by their geographic locations and by their physiography in figures 5-21 and 5-22. The ordination patterns are two-dimensional presentations of how stands compare to each other. The nearer the stands are to each other in the ordinations, the more alike they are with respect to the variables used in the comparison. As expected, species importance is closely related to geography (fig. 5-21). In the ordinations, the positions of stands in central New Hampshire merge (overlap) with those of central Vermont and Massachusetts, which in turn merge with those of Cape Cod, Long Island, and then southern New Jersey. These stands are oriented along a moisture gradient from mesic ridges and slopes of northern New England to xeric, sandy sites in New Jersey (fig. 5-22). A second moisture gradient, oriented toward ridge tops, is shown by the merging of the southern Vermont stands with slopes and then ridge stands of New Jersey, New York, and Pennsylvania. The patterns especially reflect an increasing importance of pitch pine in the sandy sites and of chestnut oak in the ridge stands. These results indicated that species importance can be used to compare stands within a given locality. The great variability in species composition encountered also denoted the difficulty of using ordinations for comparing stands outside of the original data collection area. Every time a new species IV is encountered, a new variable must be defined. Therefore, subsequent comparisons of stands with respect to their susceptibility to gypsy moth were
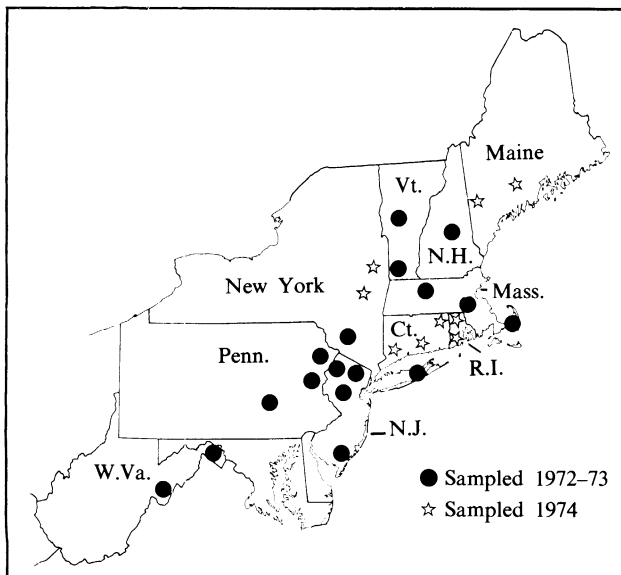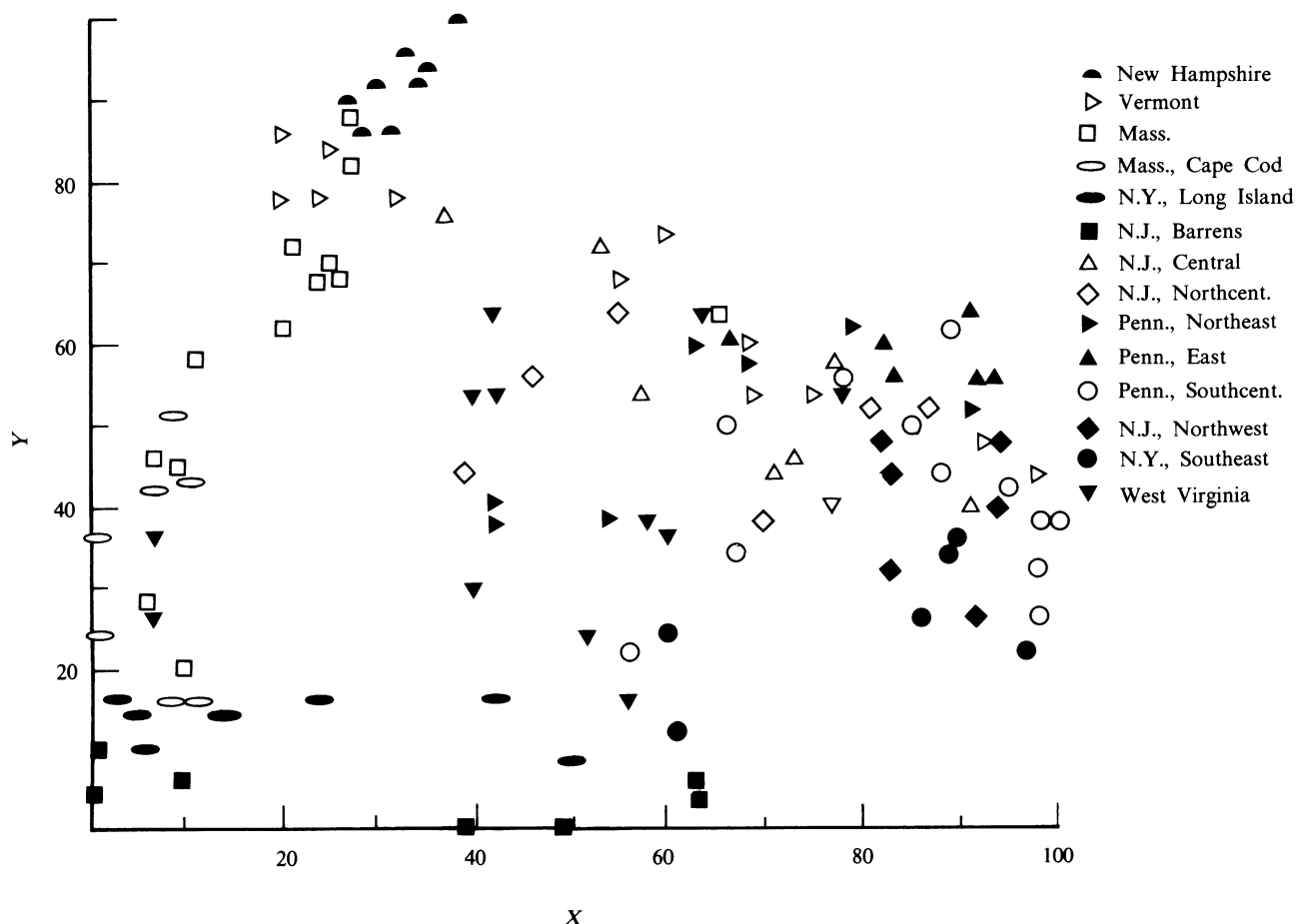
Figure 5-21.— *Principal-components analysis (PCA) of 118 forest stands based on species importance values (IV's) and labeled as to stand geography.*

Comparisons of Stands on the Basis of Tree Structural Features

made on the basis of the structural features of trees, either alone or ranked by the gypsy moth food-preference class of the species on which they occurred.

Both the B–C and PCA ordinations derived from structural features (SF's) alone produced patterns indicating that stands historically susceptible to gypsy moth were clearly different from stands historically resistant (figs. 5-23 and 5-24). In addition, when the stands' positions in the ordinations based on structural features were identified by their physiography, it

was quite clear that susceptible stands were mostly on dry ridge and sand sites, and resistant ones were often on the more mesic slopes and bottom sites (figs. 5-25 and 5-26). These results agreed with those of Bess et al. (1947). It was clear, too, that the stands on ridges and sand sites were distinctly different from each other.

Ordination Models to Classify Stands Within the Endemic Region of the Gypsy Moth

The fact that the ordinations grouped stands with similar gypsy moth susceptibilities suggested that ordination models could be used to classify stands.
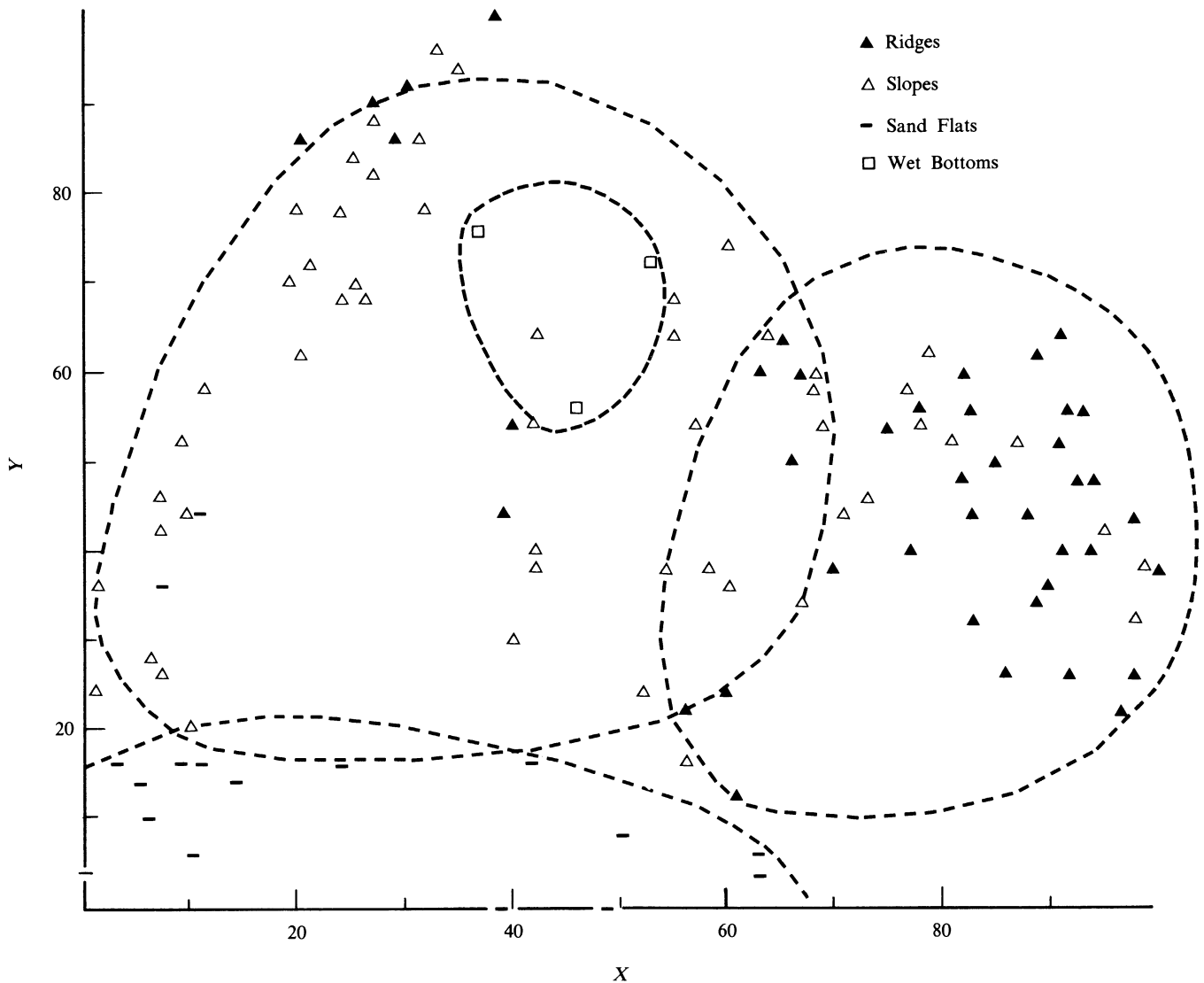
Figure 5-22.— *The same ordination as in figure 5-21, except that the stands are labeled as to their physiography. Here, and in figures 5-23 to 5-28, the contour lines arbitrarily encircle stands to clarify relationships.*

This possibility was tested in 1974 by sampling an additional 50 stands from within the old endemic region of the gypsy moth (fig. 5-20). Data from these stands were compared to spatial models derived from ordinations of the 118 stands sampled in 1972 and 1973. The models were based on PCA ordinations of the structural features ranked by the gypsy moth food-preference class of the trees on which they occurred (figs. 5-27 and 5-28). The tree species were separated into either three (SF × 3FC) or five (SF × 5FC) food-preference classes. Ordinations incorporating food-preference classes also grouped stands similar in susceptibility and physiography. A series of marker stands were determined that, when connected by a line, separated the ordination pattern into two portions. The portion above the line
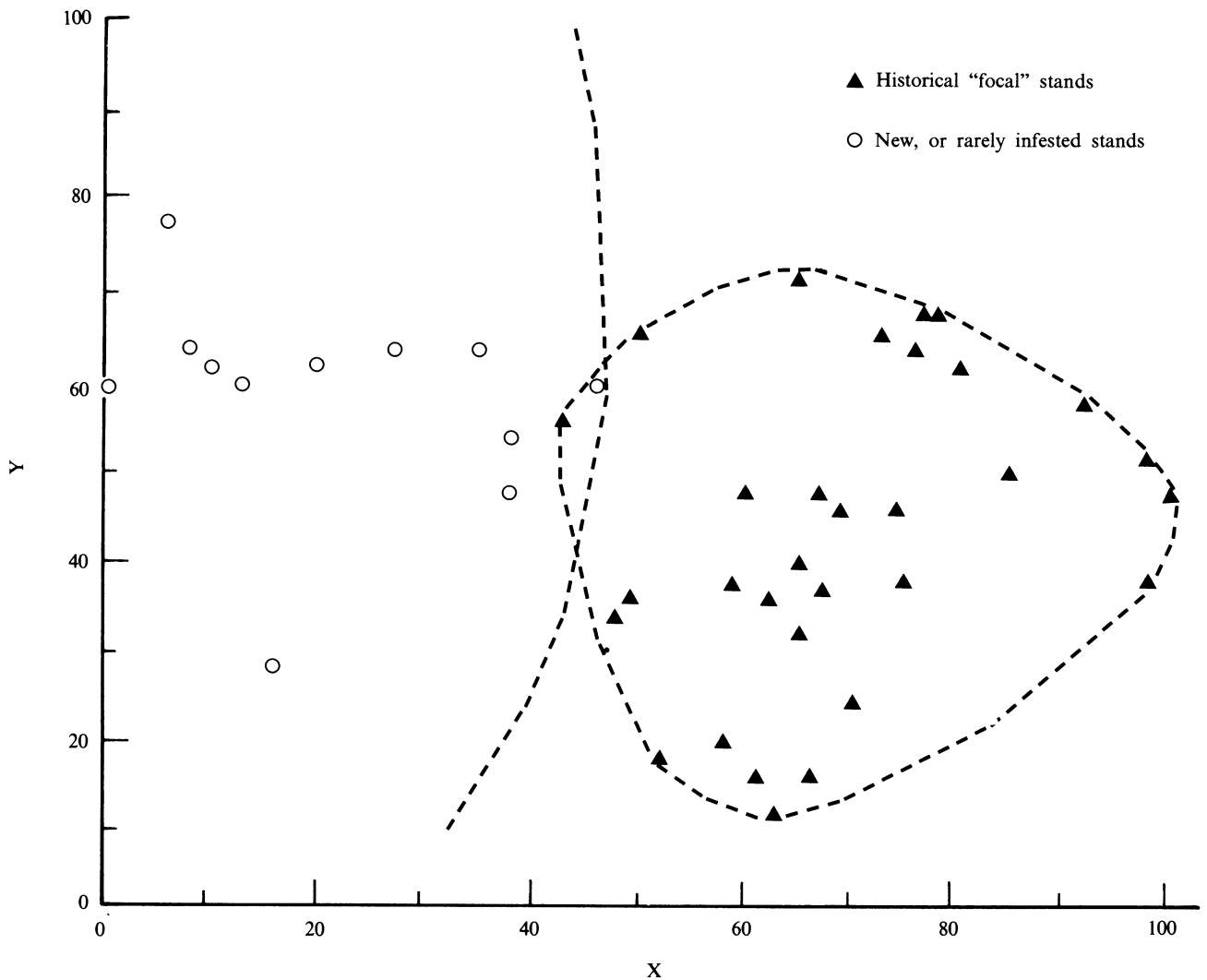
Figure 5-23.—*A Bray-Curtis (B-C) ordination of the 118 stands based on six tree-structure features and labeled as known "foci" stands; and stands that either have recently been defoliated for the first time or have been defoliated only rarely in the past. The unlabeled stands (small dots) are stands with no long-term record of defoliation.*

contained the known susceptible stands; the portion below the line contained the resistant ones. Data from the 50 stands were compared to the marker stands in the three models.

The relative positions of the 1974 test stands compared to the marker stands in the models, especially

those models incorporating host food-preference classes, corresponded well to their known defoliation histories and subjective judgement of their relative resistance or susceptibility. As an example, the classifications of 23 Connecticut stands are shown in figures 5-29 to 5-31. (Additional details and discussion of
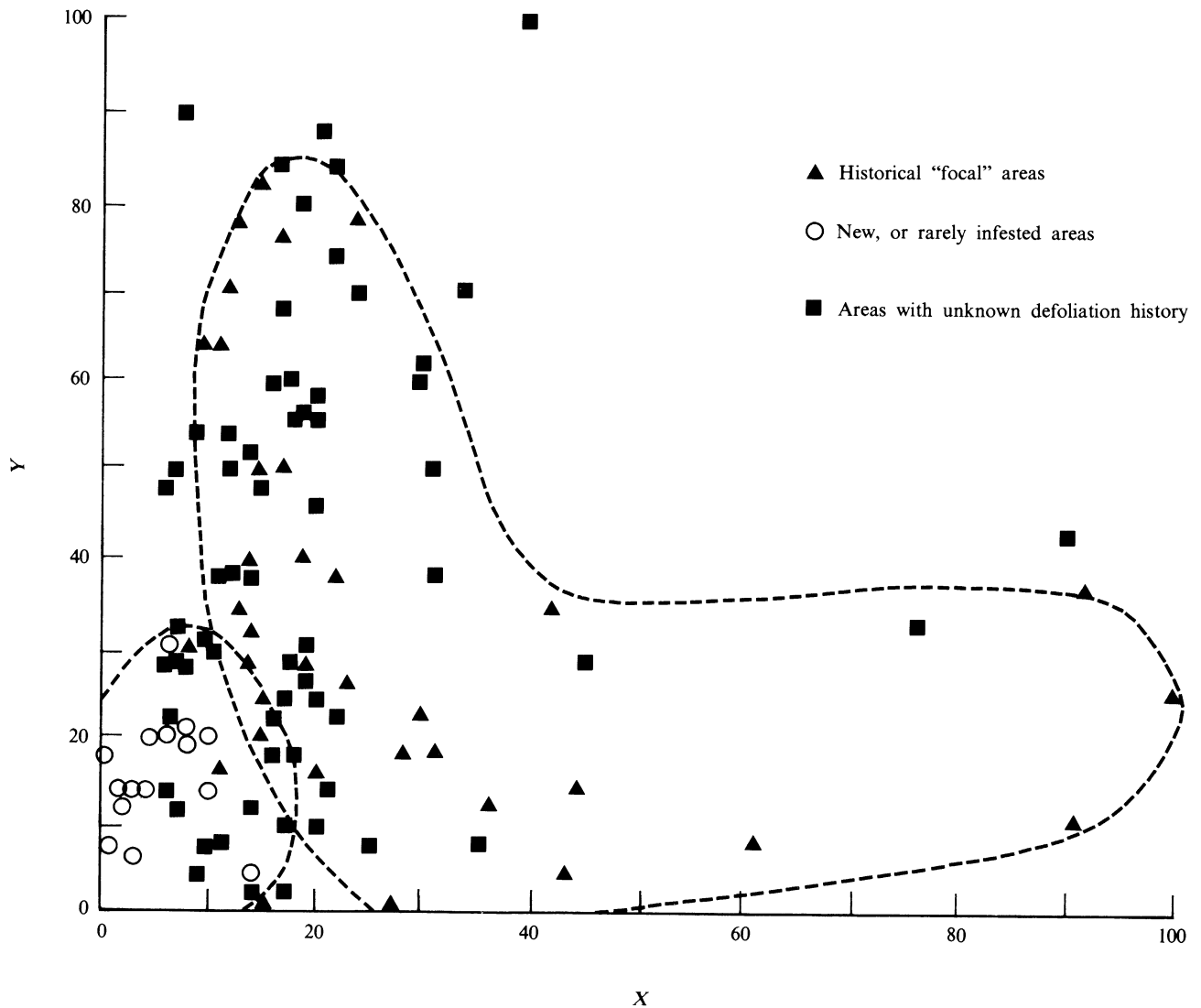
Figure 5-24.— *Principal-components ordination of 118 stands based on six tree-structure features and labeled as in figure 5-23. In this figure, stands with no record of defoliation are indicated as small squares.*

stands sampled in Maine, Rhode Island, and New York are found in Houston and Valentine 1977). In Connecticut, a special attempt was made to sample stands judged to be resistant (that is, stands with rare outbreaks that collapsed quickly). The stands sampled in southwestern Connecticut generally were defoliated severely for 1 or 2 years in the early 1970's; those in central Connecticut were similar to those in the west except that several suffered at least two severe defoliations within the past two decades (Stephens and Hill 1971), while the stands in northwestern Connecticut had not had a serious gypsy moth outbreak.
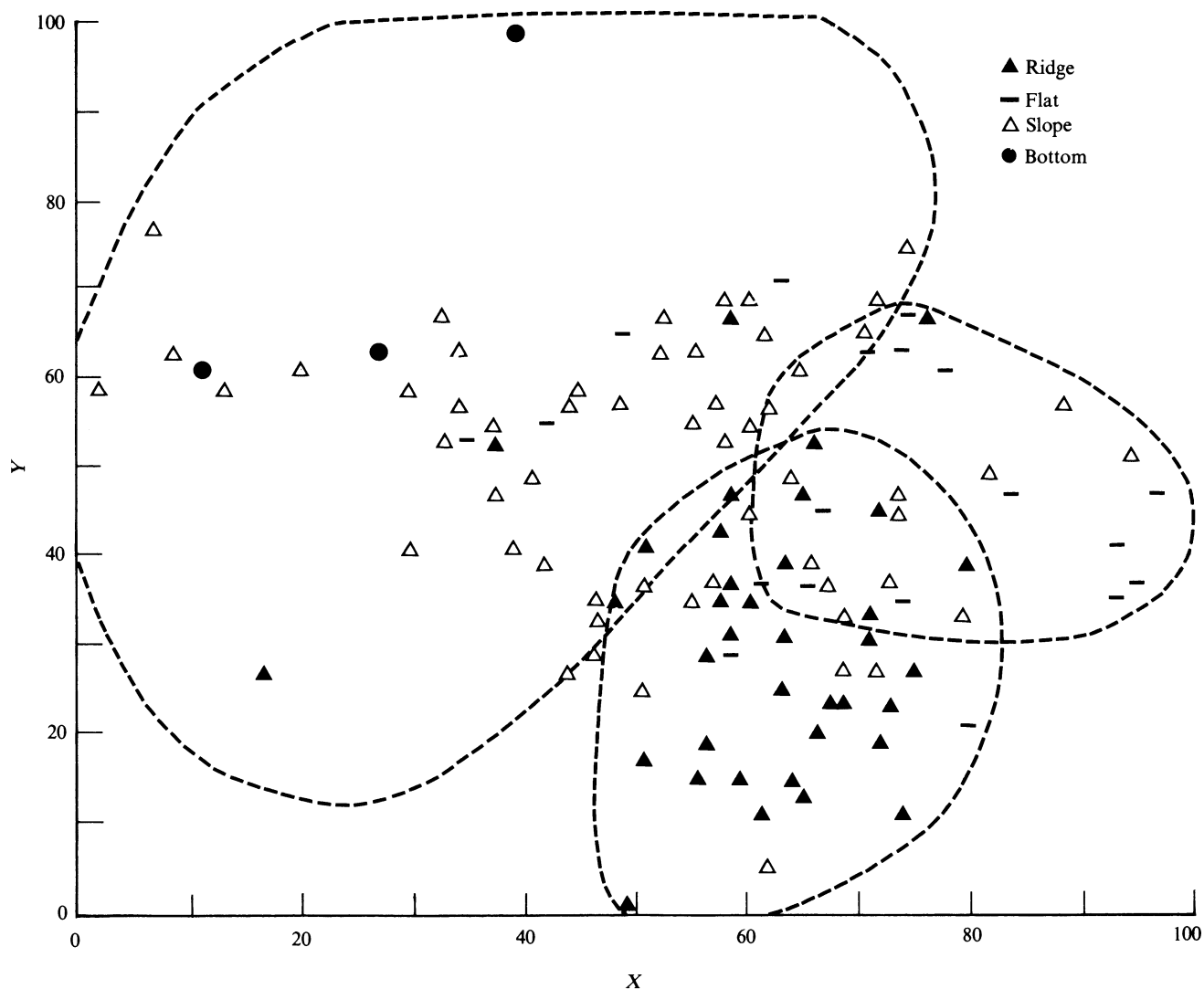
Figure 5-25.—*The same B-C ordination as in figure 5-23, with stands labeled as to their physiography.*

In the SF model (fig. 5-29) all 23 Connecticut stands are clustered tightly on the resistant side of the marker stands. Within this cluster, stands prejudged susceptible are located closest to the marker stands. In the SF × 3FC model (fig. 5-30), the stand positions better fit the knowledge of their defoliation history. Three stands appear to be susceptible. Two (E and F) are on a dry, ledgy site in central Connecticut that suffered at least two severe defoliations of several years'

duration each in the last 15 years. Stand O is in eastern Connecticut, where gypsy moth populations have been low for many years (although outbreaks did occur here in 1974).

In the SF × 3FC ordination, stands increasingly mesic, diverse in species composition, and relatively free of the measured structural features are located at increasing distances from the marker stands and decreasing distances from the origin. Similar results
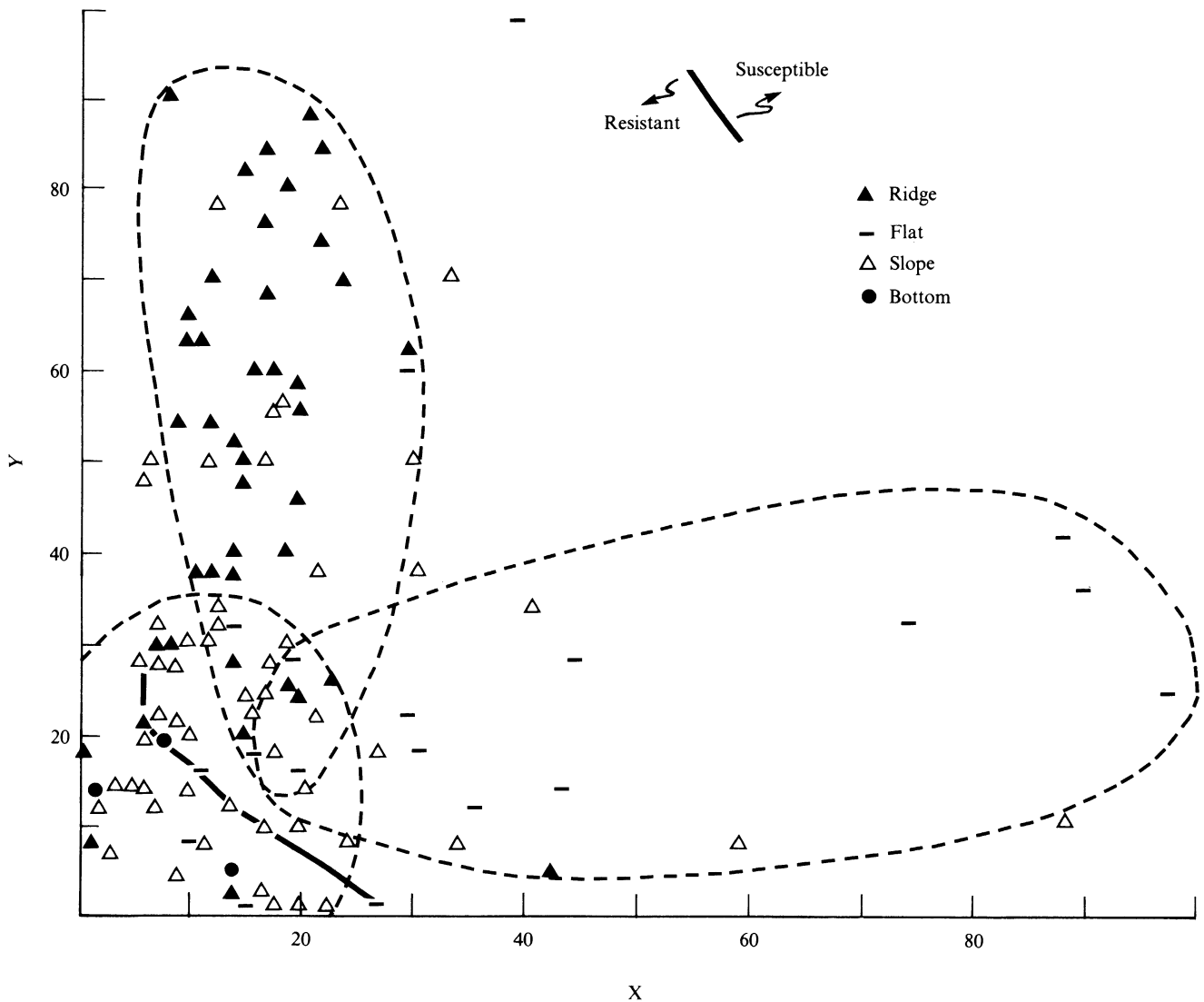
Figure 5-26.—*The same PCA ordination as in figure 5-24, with stands labeled as to their physiography. The heavy line here and in figures 5-27 and 5-28 approximately separates the ordination into susceptible and resistant stands.*

were obtained when the Connecticut stands were compared to the marker stands in the SF × 5FC ordination (fig. 5-31), except that the relative positions of certain pairs of stands were reversed—for example, E, F, Q, and T. In this latter ordination, stand T, shown barely susceptible in the SF × 3FC ordination, is

resistant, while previously resistant stand Q is now classified as susceptible. The SF × 5FC model appears to most closely match the known defoliation histories of Connecticut stands and the judgment of their relative susceptibilities based on the critera of Bess et al. (1947).

Physiography

▲ Ridges
△ Slopes
— Sand flats
□ Wet bottoms

% Oak mortality

☆ 50–100
◇ 40–49
□ 30–39
○ 20–29

Susceptible

Resistant
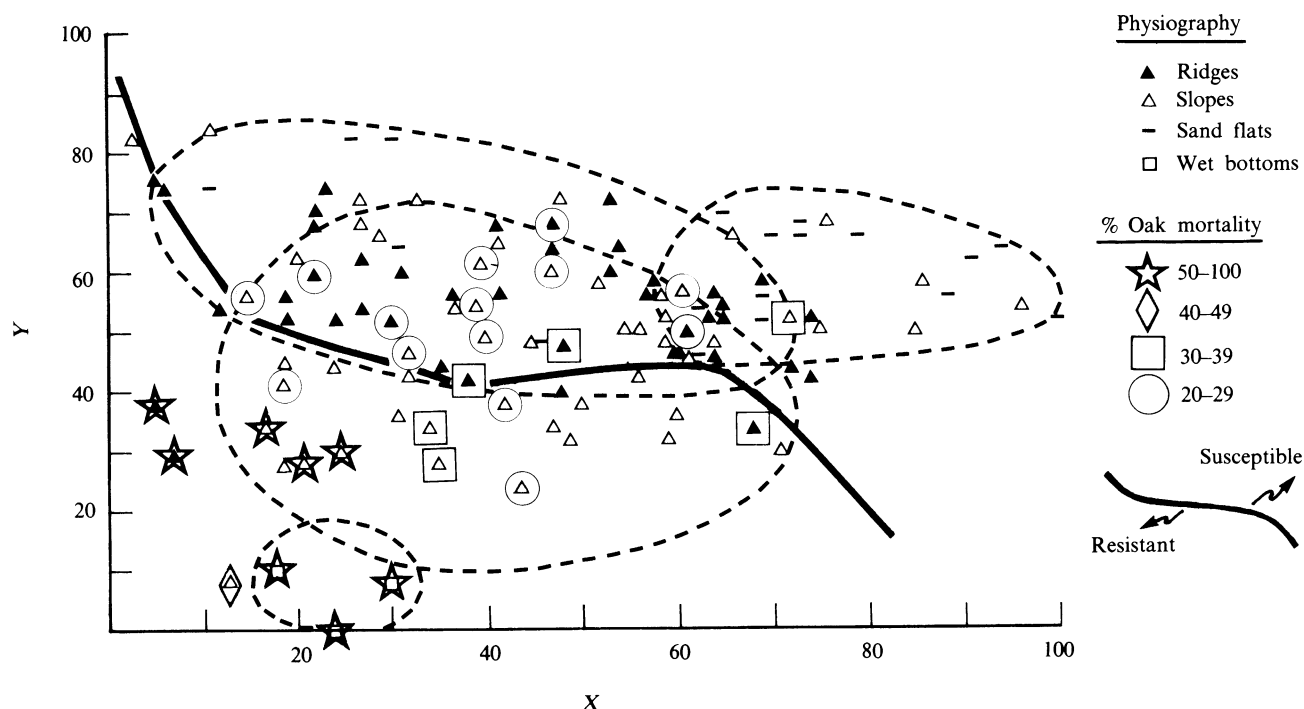
Figure 5-27.— *Principal-components analysis ordination of 118 stands based on six tree-structure features stratified by tree species separated into three gypsy moth food-preference classes. The stand locations are labeled as to their physiography and percentage of oak mortality following defoliation.*

In all cases, models taking into account the food-preference clsses of the trees bearing the structural features were better than the model using structural feature alone, and in most cases the model separating species into five food classes was best.

Ordination Models to Classify Stands Outside the Endemic Region of the Gypsy moth

The ordination models presented thus far include 13 stands from West Virginia—stands located considerable distances outside the present gypsy moth defoliation area. (Their inclusion did not affect the accuracy of the models to classify the 1974 northeastern stands.) To determine if stands outside the gypsy moth outbreak area could be compared meaningfully to those within, the West Virginia stands were classified by a PCA SF ordination of the other 105 stands sampled in 1972 and 1973 (fig. 5-32).

While in most cases the relative positions of the West Virginia stands matched subjective judgement of their potential susceptibility, several stands seemed to be misplaced (see Houston and Valentine 1977 for further details). This may reflect the generally less satisfactory classification by ordinations not incorporating host food-preference classes. It may also reflect the markedly different physiographic/moisture relationships of West Virginia compared to those in the Northeast.

In 1975, 31 stands were sampled in North Carolina, 21 on the generally very mesic Coweeta Experimental Forest near Franklin, and 10 on the Bent Creek Experimental Forest, a dry oak forest near Asheville. When compared to the SF × 3FC model, most of the Coweeta stands were classed as resistant (fig. 5-33). In
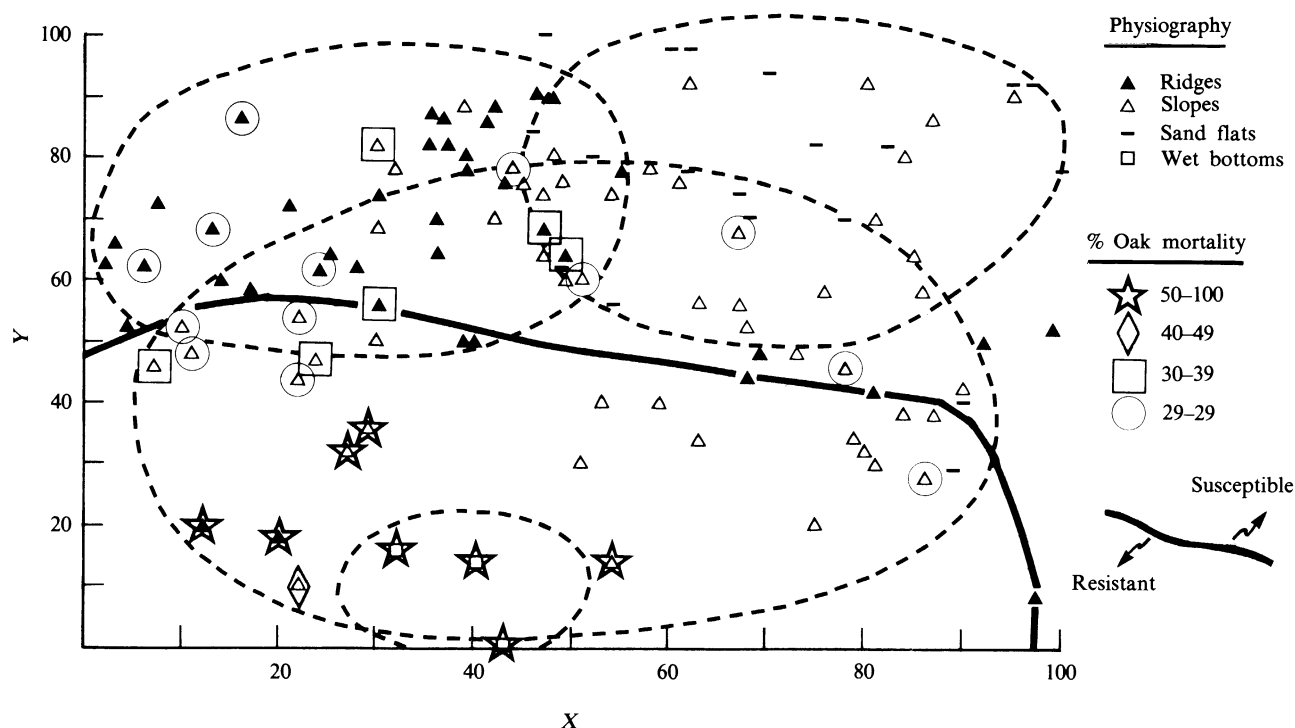
Figure 5-28.—A PCA ordination similar to that in figure 5-27. Here the tree-structure features are stratified by tree species separated into five food-preference classes (SF × 5 FC).

contrast, most of the stands at Bent Creek, when classified by the SF × 5FC model, were susceptible (fig. 5-34). These models best fitted subjective judgments of the stands' probable relative susceptibilities. Classification of the North Carolina stands demonstrated that the SF × 3FC model is most useful in classifying forests with a diversity of nonoak species, while forests rich in oak are best classified by the SF × 5FC model. This relationship was further shown when stands sampled in 1976 were compared to the two models.

In 1976, 92 forests located to the south and west of the gypsy moth outbreak area (fig. 5-35) were measured to determine how they would be classified by the model derived from northeastern forest stands: Maryland, 20; Virginia, 19; Pennsylvania, 21; Ohio, 21; and Michigan, 11.

Comparisons of these stands with either the SF × 3FC or SF × 5FC models are shown in figures

5-36 to 5-41. To aid in this comparison and to demonstrate how the stands differ from each other, brief descriptions are given for all the stands measured in Maryland (table 5-22) and Michigan (table 5-24), and for a few representative stands in Pennsylvania, Ohio, and Virginia (table 5-23).

In Maryland (fig. 5-36, table 5-22) both the SF × 3FC and SF × 5FC models classified stands as expected on the basis of previous studies. Most stands on adverse sites, such as those on the deep sands of Elk Neck Park and on the dry ridges of Rocks State Park, were classed as susceptible, while most stands on mesic sites were rated resistant. The central and southern Pennsylvania stands are compared in figures 5-37 and 5-38 and in table 5-23. Generally, the position of the stands in the models fitted well with subjective judgments of their relative susceptibilities, dry, rocky ridge tops (for example, stand D, fig. 5-37) being more susceptible than their lower slope counterparts (stands
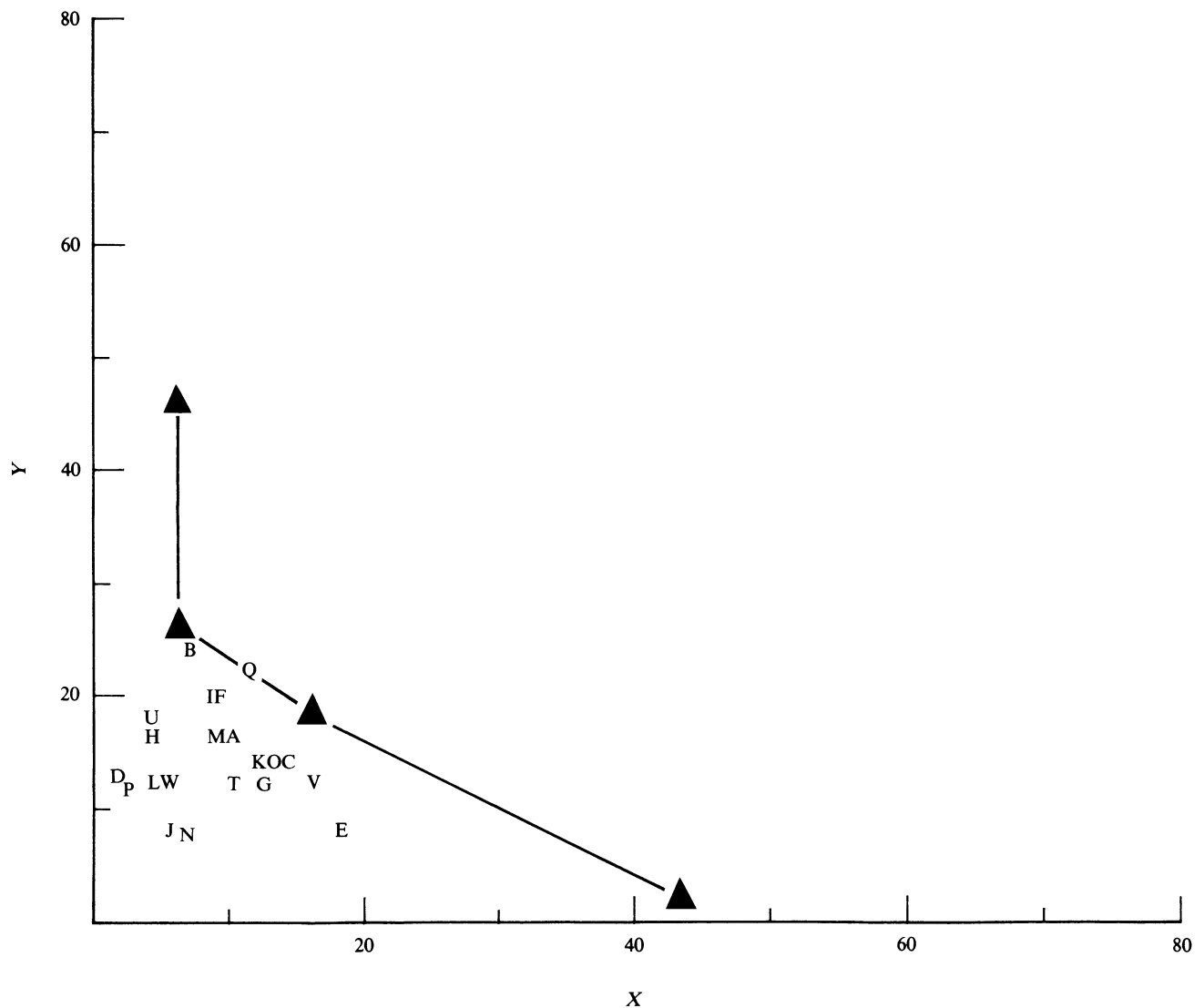
Figure 5-29.—*A PCA ordination of 118 stands based on tree-structure features. Here and in figures 5-30 to 5-34 and 5-36 to 5-41, only the marker stands along the line that separates the ordination into susceptible (upper and right) and resistant (lower and left) portions are shown. The letters locate 23 Connecticut stands in relation to the marker stands.*

E and *F*, fig. 5-37, table 5-23). Interestingly, most of the stands in Somerset and Bedford Counties except for two quite dry sites, (fig. 5-38, table 5-23) were classed as resistant. This is the area where an early outbreak occurred around a dump site in 1970 and

where early trials had been run with the sex pheromone disparlure. The insect populations soon crashed in these stands and have remained at low levels since. The Virginia stands (fig. 5-39, table 5-23) located on the Shenandoah National Park and on the
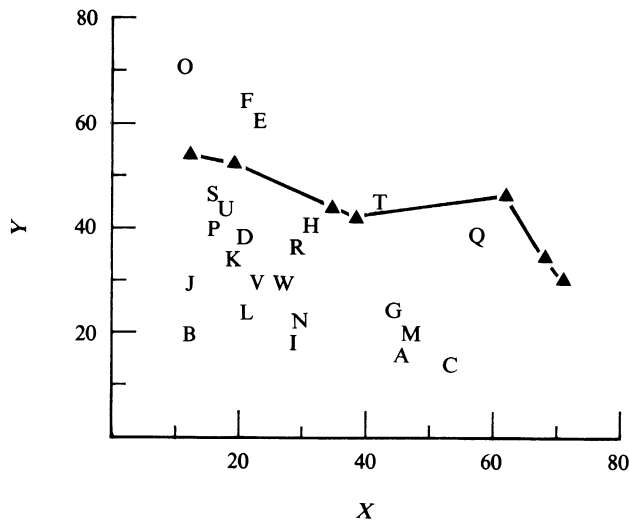
Figure 5-30.—*A PCA ordination similar to figure 5-29, except that the SF variables are stratified by three food-preference classes (SF × 3 FC).*
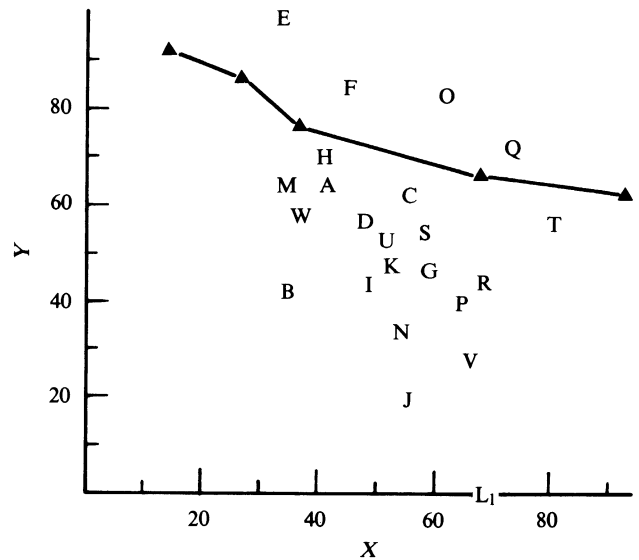
Figure 5-31.—*A PCA ordination similar to figures 5-29 and 5-30, except that the SF variables are stratified by five food-preference classes (SF × 5 FC).*

George Washington National Forest were mostly dry oak stands on ridges or upper slopes and were classified as susceptible. The few mesic rich woods (stands *F, Q,* and *O*) were classed as resistant. In Ohio, (fig. 5-40, table 5-23) stands sampled in the southeastern part of the State (stands *A-K*) ranged from dry upper slopes, classed as susceptible (for example, stand *E*), to mesic slopes classed as resistant (for example, stand *D*). All but one of the woodlots of northwestern Ohio (stands *L-U*) were classified as quite resistant. The one slightly susceptible stand (stand *P*) was a grazed, mesic-to-dry flat field site especially rich in oak.

Finally, the Michigan stands are compared with the SF × 3FC model in figure 5-41. As described in table 5-24, many of the Michigan stands were quite different from those sampled in other States. Most stands in Midland and Clare Counties were growing on sandy, flat sites with slightly depressed wet areas. Most stands contained species characteristic of early successional stages, sometimes in abundance. Some stands rich in aspen (for example, stands *A* and *B*) were classed susceptible by the SF × 3FC model and quite resistant by the SF × 5FC model. Bess et al. (1947)
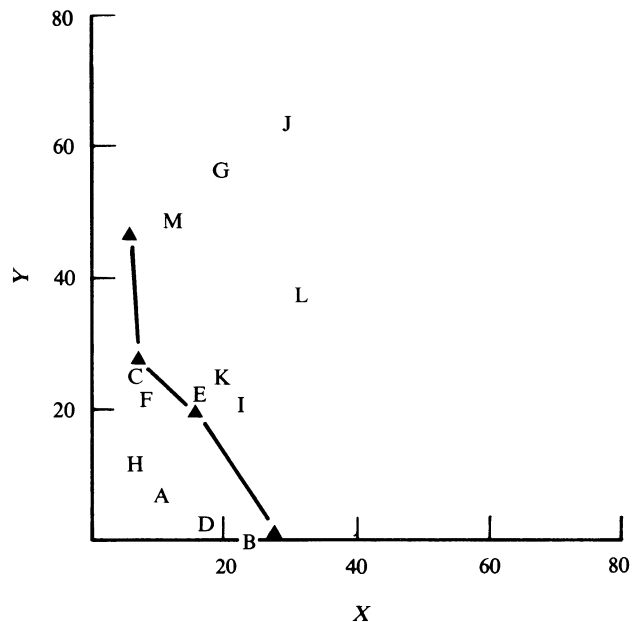
Figure 5-32.—*A PCA ordination of 105 stands based on six SF variables. The letters shown the location of 13 stands in West Virginia compared to the marker stands.*
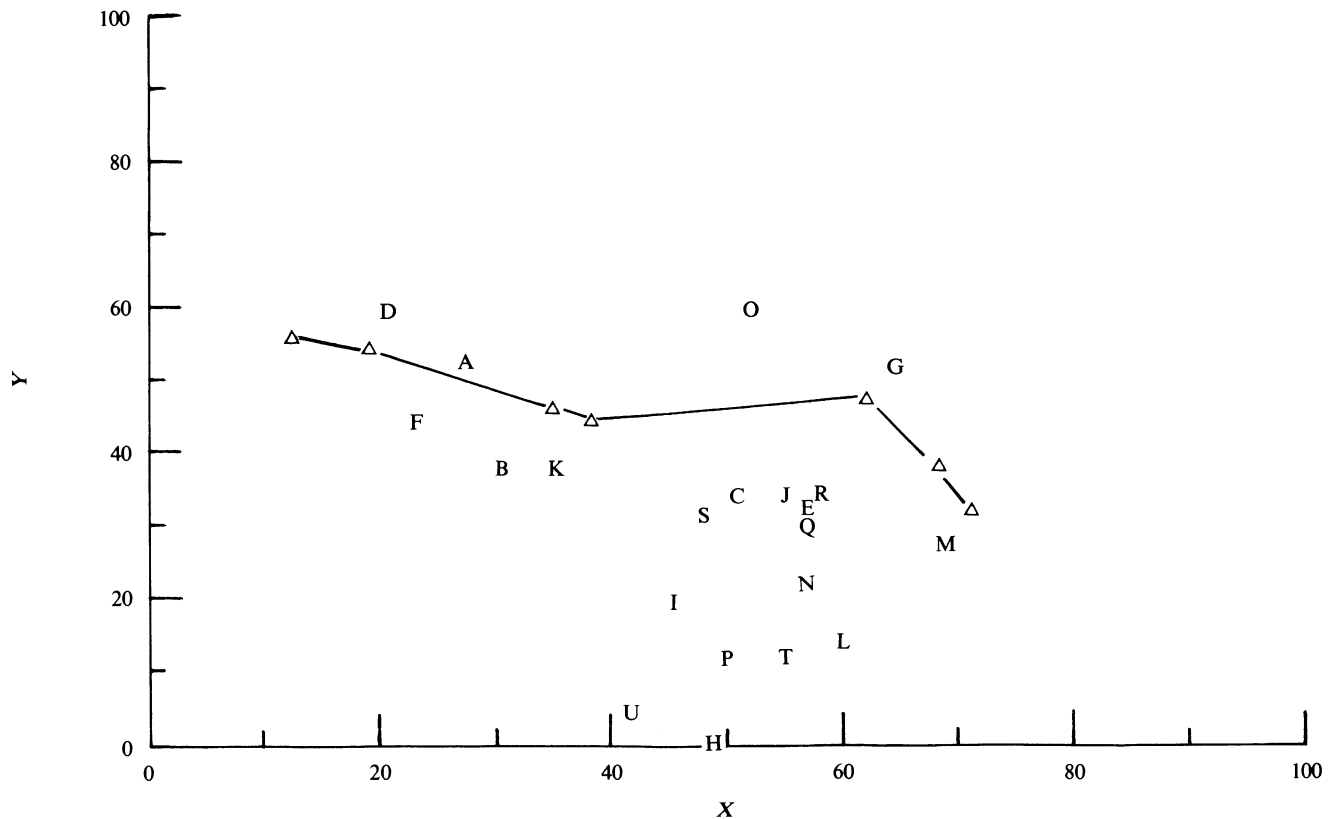
Figure 5-33.—*The same PCA ordination of 118 stands as in figure 5-30, except that the letters locate 21 stands on the Coweeta Experimental Forest in North Carolina.*
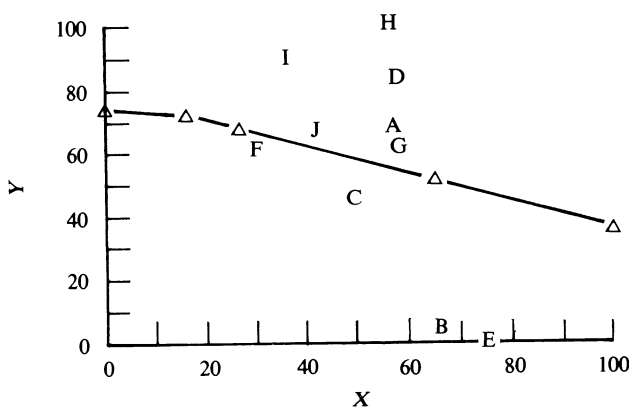
Figure 5-34.—*The same PCA ordination as in figure 5-31, except that the letters show the locations of 10 stands on the Bent Creek Experimental Forest in North Carolina.*

considered New England stands high in aspen, gray birch, and early successional stage oaks to be highly susceptible. Therefore, the SF×3FC model, which places these species in food class 1, is the model best suited to classify stands rich in these early successional species.

The results suggest that States immediately to the west and south of the present outbreak area possess forests ranging in susceptibility from highly susceptible to highly resistant. It would appear, then, that barring an adverse effect by climate or unsuspected predators and parasites, the gypsy moth will encounter many favorable forests in its southwestward trek.

The ordination models described here are preliminary ones, but the apparent accuracy with which they
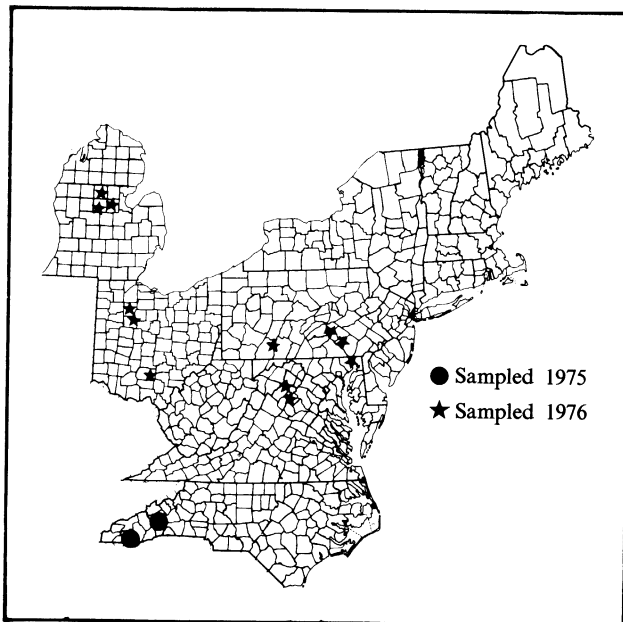
Figure 5-35.—*Areas where forests were sampled in 1975 and 1976. These forests, except for some in Pennsylvania, are located to the south and west of the current outbreak area.*

Figure 5-36.—*A PCA ordination similar to figure 5-31, except that the letters locate 20 stands in Maryland.*

classify stand susceptibility suggests that their use should be evaluated further.

Tests should be conducted, along the advancing front of gypsy moth outbreak, to evaluate the effectiveness of the models in determining stands where outbreaks will occur, where monitoring of insect populations should be continued, and where preoutbreak control actions should be centered. In addition, the use of these techniques to identify future outbreak foci should be tested in those areas where the initial outbreak wave has passed through.

To use the models in their present form considerable time is needed to acquire the necessary data, and relatively sophisticated computer facilities are needed to perform the ordinations. Studies are now underway to simplify the data acquisition process, and canonical analysis models are being developed that will permit the assigning of probabilities to predictions of stand susceptibility or resistance. Once these goals have been achieved, models predicting

stand susceptibility will become an important part of a gypsy moth management system.

Mortality and Factors Affecting Disease Development

David R. Houston

While it is true that many trees die after they are severely and repeatedly defoliated by gypsy moth, it is also true that many trees do not die. The number of trees that succumb after a particular defoliation episode can vary markedly from stand to stand, and within a given stand mortality may vary greatly from episode to episode. The factors responsible for this variation have been difficult to determine. Often, correlations between mortality and certain site variables that exist for one case are not important elsewhere or at other times. Stand and site variables, all complex and interelated, that seem most important biologically are abundance and condition of organisms of secondary action, the frequency and severity of defoliation, the occurrence of environmental stress and disturbance prior to and during defoliation, the condition of the trees prior to defoliation, and the species composition and physiography of the stand.

Table 5-22.—*Brief descriptions of Maryland stands (fig. 5-36)*

Letter	Location	Physiography/moisture	Stand description
A	Cecil County (Dupont Estate)	Mesic, steep	Mixed stand of very large red, black and white oak, tulip poplar, beech and hickory.
B	(Dupont Estate)	Slope less steep and more mesic than A	Stand similar to stand A but with fewer oak.
C	(Egg Hill)	Dry, hill top	A fairly open stand with large chestnut oaks predominating. Some black and scarlet oak and sassafras.
D	(Egg Hill)	Mesic, flat	Stand more open than stand C with large white oak, tulip poplar, beech, blackgum, and mockernut hickory.
E	(Elk Neck State Forest)	Dry-mesic, hill top	Stand varies from xeric areas with small chestnut and black, scarlet and blackjack oak to more mesic areas with red maple, dogwood, black cherry, and shadbush.
F	(Elk Neck State Forest)	Dry sand flat	Stand with small chestnut, black and scarlet oak, and Virginia pine.
G	(Elk Neck State Forest)	Dry, flat	Stand with small trees: black, scarlet, and white oak, sassafras, big tooth aspen, sweetgum, and Virginia and pitch pines.
H	(Elk Neck State Forest)	Dry, flat	Similar to stand G but oak and sweetgum more important.
I	Cecil County	Moderately mesic, flat	Isolated woodlot. A variable stand with areas of even-aged scarlet, black, white, and blackjack oak, and sweetgum; open areas with wolf-trees; and areas with large, tall white, black, and scarlet oak, sweetgum, and hickory.
J	Cecil County	Mesic-dry, flat	An all-aged stand of black, scarlet, and white oak, blackgum, and sweetgum, with some mockernut hickory, beech, red maple, and black cherry.
K	Harford County	Mesic, slope	An even-aged stand with poorly stocked overstory of chestnut, white, black, and scarlet oak, and hickory and an understory of dogwood and blackgum.
L	Harford County	Mesic, flat	An isolated woodlot, even-aged, older than stand K, and more open overstory of chestnut, white, and black oak. Sassafras, blackgum, hickory, and maple occur in the understory.
M	(Broad Creek Mem. Scout Reservation)	Dry-mesic, upper slope	An all-aged stand with tall white, chestnut, black, and red oak. Hickory, red maple, beech blackgum, and oak are common in the understory.
N	Broad Creek Mem. Scout Reservation)	Dry-mesic, ridge	A stand with trees shorter and more wolflike than in stand M; overstory species composition similar but with no red oak.
O	(Rocks State Park)	Mesic, lower slope	A stand with large, tall red, chestnut, and white oak and tulip poplar.
P	(Rocks State Park)	Dry, upper ridge	An open stand with short, crooked chestnut and black oak and hickory.

Table 5-22.- *(cont.)*

Letter	Location	Physiography/ moisture	Stand description
Q	(Rocks State Park)	Dry, mid-slope, slope, rocky	Similar to stand *P* but with scarlet oak as well. Some red maple and blackgum occur in the understory.
R	(Rocks State Park)	Mesic, dry slope	A stand more densely stocked with taller trees than stand *Q*; species composition is the same but includes sassafras and oak in the understory.
S	Harford County	Mesic, flat	A rich woods with very tall, large red, black, and white oak and tulip poplar.
T	Harford County	Mesic, flat	A stand similar to stand *S*.

Table 5-23.—*Brief descriptions of some representative forest stands from Pennsylvania, Virginia, and Ohio (figs. 5-37 to 5-40)*

Letter	Location	Physiography/ moisture	Stand description
	Pennsylvania (fig. 5-37)		
D	Dauphin County (Peters Mtn.)	Dry, rocky, ridge top	Stand has large oaks, many wolf trees.
E	(Peters Mtn.)	Dry-mesic mid-slope, rocky	Stand contains some white oak and tulip poplar along with other oak species.
F	(Peters Mtn.)	Mesic, lower slope	In this stand, trees are larger and taller than in stand *E* with oak, tulip poplar, and red maple predominating.
H	Lancaster County	Dry knoll and steep, south-facing slope	A stand with many scraggly chestnut oak and blackgum wolf trees.
J	Lancaster County	Very mesic, lower slope	A stand with large tulip poplar, black birch, red maple. Chestnut, black, red, and white oak present but not predominant.
K	Lancaster County	Mesic, ridge top	A stand with many large chestnut, black and scarlet oak, blackgum, tulip poplar, sassafras,and mockernut hickory. There is evidence of fire in many areas.
	(fig.5-38)		
F	Somerset and Bedford Counties	Mesic, flat	A stand with many tall red and black oak, red maple, black birch, cucumber trees, and a few pitch pines.
F	Somerset and Bedford Countries	Dry-mesic flat	Part of this stand contains tall red oak, red maple, and black cherry, and large shadbush and sassafras.

Table 5.23. - *(cont.)*

Letter	Location	Physiography/moisture	Stand description
I	Somerset and Bedford Counties	Dry-mesic steep rocky slope	A fairly open stand with large red and chestnut oak shagbark, pignut, and red hickory.
	Virginia (fig. 5-39)		
C	Shenandoah National Park	Dry, south-facing slope	An open stand with moderate-size red and white oak, some of which are wolf trees.
D	Shenandoah National Park	Dry-mesic, slope	A dense stand with tall red and white oak with some red maple, black birch, and hickory.
F	Shenandoah National Park	Mesic, north-facing slope	A richer wood with tall red oak, black birch, and hickory and a lush ground cover.
K	George Washington National Forest	Xeric, ridge	A fairly dense stand of short, scaggly chestnut oak and pitch pine trees with sparse canopies.
P	George Washington National Forest	Dry, slope	An open stand with moderate-size black, white, and red oak, pignut hickory, and some Virginia pine.
Q	George Washington National Forest	Wet-mesic, near stream bottom	A stand of large white oak and pine, with tulip poplar, red maple, and dogwood as a midcanopy.
	Ohio (Southeast) (fig. 5-40)		
A	Ross County (Tar Hollow State Forest)	Dry-mesic, upper slope	Dry areas of this stand have tall chestnut oak, sassafras, red maple, sugar maple, and hickory; mesic areas have tulip poplar, black walnut, elm, and black locust.
D	(Tar Hollow State Forest)	Mesic, upper slope	The overstory of this stand is oak, tulip poplar, and hickory; the understory contains hickory, ash, elm, and dogwood. Oak is not predominant, and the ground cover is lush and diverse.
E	(Tar Hollow State Forest)	Dry, upper slope	The stand has areas with tall chestnut, scarlet, black, red, and white oak. In other areas, trees are smaller.
	Ohio (Northwest)		
P	Hardin County	Mesic-dry, flat	A stand with light stocking of oak, ash, and hickory. This stand which has been grazed, is the "oakiest stand in northern Ohio."
R	Hancock County	Wet-mesic, flat	A pole-size stand of hickory with red, white, and swamp white oak, white ash, ironwood, and elm.
T	Hancock County	Wet-mesic, flat	A stand predominantly of tall red, white, and swamp white oak with admixes of basswood, bitternut hickory, elm, red maple, sugar maple, beech, and white and blue ash.

Table 5-24.—*Brief descriptions of the Michigan forest stands (fig. 5-41)*

Letter	Location	Physiography/ moisture	Stand description
A	Midland County	Dry, flat	An open stand of small northern pin oak, aspen, pine, and black cherry, with an occasional large oak, and a dense bracken ground cover.
B	Midland County	Dry-wet, flat	A scrubby stand of northern pin oak, black cherry, aspen, and pin cherry with openings. The aspen is mostly overmature and in poor condition.
C	Midland County	Dry-wet, flat	A stand more scrubby and open than stand B, with small northern pin, red, and white oak, aspen, red maple, black cherry, and white birch.
D	Midland County	Dry, flat	A scrubby stand of small red and northern pin oak, pine, red maple, and white birch and a heavy reproduction of pine in the understory.
E	Isabella County	Dry-mesic, slope	A variable stand: One area with tall, slender trees, other with larger trees. Species present include red and white oak, red maple, aspen, cherry, white ash, and white birch.
F	Isabella County	Mesic, slope	A stand similar to but younger and more open than stand E.
G	Isabella County	Mesic-dry, sandy, flat	An even-aged stand with large white pine, red and white oak, black cherry, red maple, beech, and hemlock.
H	Clare County	Dry-mesic, flat	Much of this stand is open and parklike with northern pin and black oak, and aspen. Some areas bear red maple and white oak.
I	Clare County	Wet-dry, flat	Trees in this stand are mostly large black cherry, white birch, aspen, red maple, red oak, white oak, and a few northern pin oak.
J	Clare County	Dry, flat; mesic, knoll	A parklike stand of northern pin and white oak, and aspen on the flat areas, and red maple, white birch, and red oak on a knoll.
K	Clare County	Dry, flat	A parklike stand with a sparse canopy of jackpine, northern pin, black, and white oak, and aspen.

Organisms of Secondary Action

The presence of both a root-attacking fungus, *Armillaria mellea*, and the twolined chestnut borer, *Agrilus bilineatus*, has long been associated with oak mortality following gypsy moth defoliation. Baker (1941), who recognized that the death of many "thrifty" trees following the early New England epizootic might not be due to defoliation alone but to attack by these organisms, noted that an outbreak of *A. bilineatus* occurred from 1912 to 1915. Many of the Melrose Highlands study plots were abandoned during this period because of excessive tree mortality.

Indeed, only 122 of the original 264 plots established in 1911 were followed until 1921 (Campbell and Sloan 1977). Although specific reasons for such high mortality (62 percent of the oaks in the abandoned plots) are not given, it is likely that severe attacks of the defoliated trees by *A. bilineatus* and by *A. mellea* were primary factors.

As part of a detailed study on *A. bilineatus* in New York and Pennsylvania, Côté (1976) examined oaks that had died the previous year for the presence of *A. mellea* and *A. bilineatus* following gypsy moth defoliation. On the basis of the presence and/or absence of rhizomorphs or mycelial fans at the root
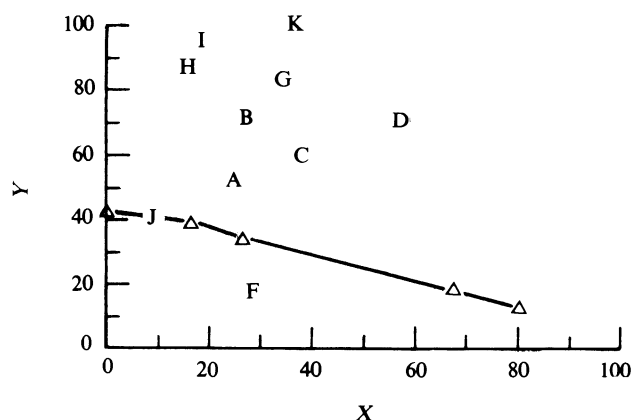
Figure 5-37.—*The same PCA ordination as in figure 5-36, except that the letters locate 10 stands in Dauphin and Lancaster Counties, Pa.*
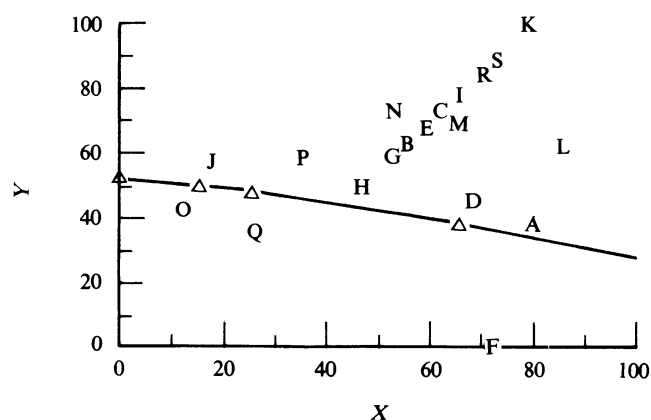
Figure 5-39.—*The same PCA ordination as in figure 5-36, except the letters show the positions of 19 stands in Virginia on the Shenandoah National Park (stand A-G) and the George Washington National Forest (stands H-S).*
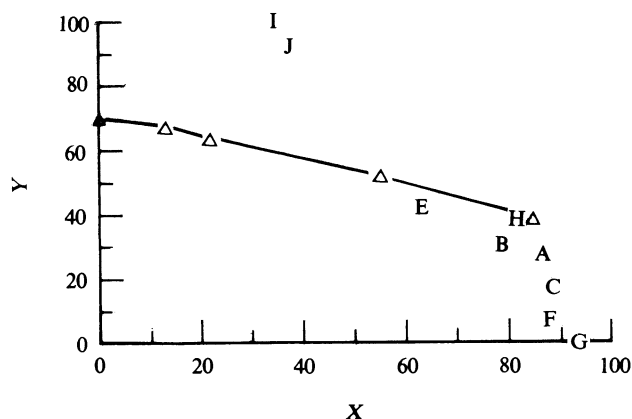
Figure 5-38.—*The same PCA ordination as in figure 5-36, except that the letters show the locations of 10 stands in Bedford and Somerset Counties, Pa.*
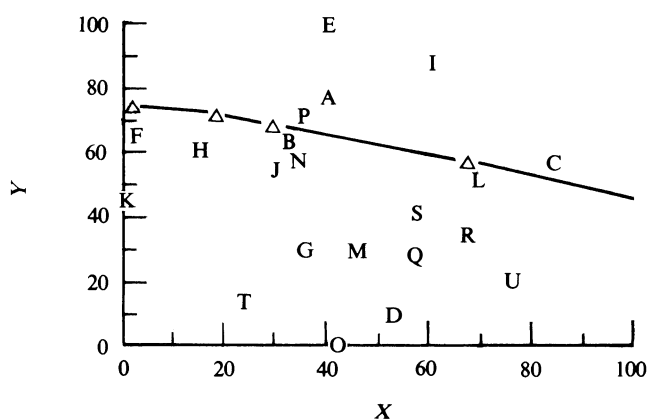
Figure 5-40.—*The same ordination as in figure 5-36, except that the letters locate the positions of 21 stands in Ohio (stands A-K were in southeastern Ohio forests, stands L-U were in northern Ohio woodlots).*

collar (and sometimes on one root up to 1 m from the root collar), he concluded that *A. bilineatus* contributed much more to tree mortality than did *A. mellea.* Similar results were obtained by Dunbar and Stephens (1975), who examined recently dead oaks in Connecticut for the presence of these organisms. Observations for *A. mellea* were limited to the root-collar area. On this basis, these workers assigned a

minor role to the fungus and a major role to the insect in the death of defoliated trees. Kegg (1971, 1973) reported that defoliated trees that wilted and died in late summer and fall had evidence of *A. mellea* and were attacked by *A. bilineatus.*

The twolined chestnut borer was also responsible for killing attacks on the red oak group in Pennsylvania following defoliation by the oak leaf
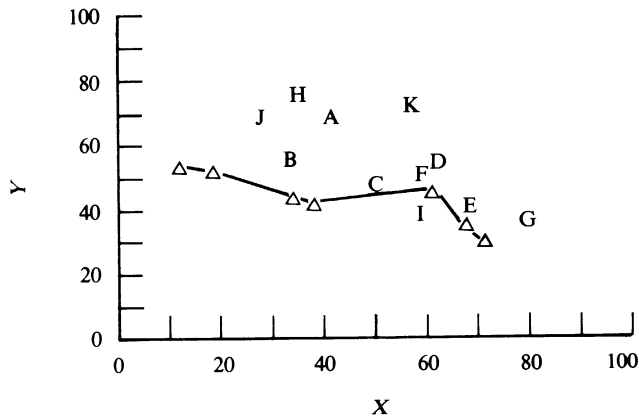
Figure 5-41.—*A PCA ordination similar to figure 5-30, except that the letters locate the positions of 11 stands in Michigan.*

roller, *Croesia semipurpurana* (Nichols 1968). In an earlier report on this problem, Staley (1965) concluded that both *A. bilineatus* and *A. mellea* were secondary mortality agents—and that *A. mellea*, when it did occur, was only weakly pathogenic.

The role of secondary-action organisms has always been difficult to assess. *A. mellea* has been classed as an aggressive primary pathogen capable of successfully attacking and killing vigorous hosts; as a saprophyte capable of attacking only tissues either dead or moribund; and as a facultative pathogen that, given the opportunity, can attack and kill trees altered by an adverse environmental factor such as defoliation. It is probable that each of these diverse opinions is correct—with different expressions of pathogenicity the consequence of different hosts interacting with different inoculum loads and strains of the fungus, coupled with an often incomplete knowledge of prior stresses.

Some early observations and several recent field studies conducted specifically to clarify the role of *A. mellea* in the death of defoliated trees indicate that this organism is more important than shown by the earlier studies.

Several studies show that mortality of defoliated sugar maples is associated with the presence of *A. mellea*. For example, in one study no sugar maple

saplings died even where they had been artificially defoliated for 3 consecutive years (Parker and Houston 1971). In this young stand where sugar maples had seeded directly onto an abandoned, previously tilled field, no woody food bases for *A. mellea* occurred nor were there rhizomorphs of the fungus on tree roots or in the soil.

In contrast, when saplings were inoculated with *A. mellea* in a nearby young sugar maple stand, some saplings died after just one defoliation (Wargo and Houston 1974). Indeed, in this study three of the four defoliated but noninoculated trees that died were attacked heavily by *A. mellea*, which emanated from naturally occurring food bases.

These results suggest that stand history may be important not only as it affects tree susceptibility but also as it affects the populations of agents of secondary action. For example, in one area near Livonia, Pa., defoliation in the 1960's by oak leaf rollers triggered severe oak decline and mortality in red and scarlet oaks (Staley 1965). A recent study of oak decline following gypsy moth in the same area revealed that *A. mellea* was more important than apparently it had been after the leaf roller outbreak. It is probable that the fungus had become established on many trees as a consequence of the previous attack (Wargo 1977a) and it is likely that the root systems of trees killed in the 1960's provided many favorable food bases for *A. mellea*, thus enhancing its establishment in the trees predisposed by the recent gypsy moth outbreak.

A second important point disclosed by this study was that the occurrence of *A. mellea* at the root collar in recently dead trees is a poor indicator of whether the fungus is colonizing the root system (Wargo 1977a).Based solely on this criterion, the organism's importance can be seriously underestimated.

With respect to cause/effect then, oak decline and mortality must be viewed as a complex. It is initiated by the drastic insult of defoliation, is continued as the tree responds biochemically and as opportunistic organisms invade, and is completed as the invaded tree is girdled and overcome by *A. mellea, A. bilineatus,* or both. Whether one or both of these organisms become important following a defoliation episode

depends on factors that affect their population levels and conditions, as well as on factors that affect the host tree. Some of these factors, including defoliation regimen, tree condition, site factors, and species composition, are discussed briefly in the following paragraphs.

Defoliation Regimen

When enough leaves are removed from a tree enough times, the tree will decline and often will die. It should be clear from earlier sections in this chapter that the relationship between defoliation and a tree's death hinges around 60 to 75 percent. Data from the Melrose case study have shown clearly that defoliation levels of less than this amount, even when repeated annually for as many as 5 years, result in relatively little tree mortality (Campbell and Valentine 1972).

But defoliation heavy enough to trigger refoliation and the attendant internal biochemical changes discussed earlier can initiate dieback and decline and lead to tree death. Not only is the number of trees that die during a given period of time increased, but more importantly, the pattern of death is affected. Normally, suppressed understory trees in poor condition die and drop from the stand gradually as the forest undergoes development over time. But heavy defoliation, especially when repeated, accelerates the death of understory suppressed trees and in addition can result in death of dominant trees, even those in good condition.

The magnitude of tree mortality is related to the number of years of successive heavy defoliation. Over a decade, a single heavy defoliation has relatively little effect on tree mortality and on stand structure, but 2 or more years of heavy defoliation can trigger significant mortality of dominant trees and markedly affect stand composition and structure (Campbell and Sloan 1977, Stephens and Hill 1971, Baker 1941, Campbell and Valentine 1972, Kegg 1973).

Table 5–25 illustrates the increase in mortality that accompanied increasingly severe defoliation regimes in the Melrose Highlands plots.

Tree Condition

The Melrose data show clearly (table 5–26) that trees in "fair" and especially those in "poor" condition prior to heavy defoliation were much more likely to die than "good" trees (Campbell and Valentine 1972, Campbell and Sloan 1977). This relationship has been noted by others, although Nichols (1968) found no consistent correlation of "relative vigor" prior to defoliation to decline and mortality in Pennsylvania. Recent analysis of data collected in northeastern Pennsylvania during the Expanded Gypsy Moth Program revealed that tree condition prior to de-

Table 5–25.—*Percent cumulative mortality among mixed oaks of all size and crown classes following 1, 2, or 3 successive years of heavy defoliation*[1]

Years after last heavy defoliation	Percent cumulative mortality after number of years of heavy defoliation		
	1	2	3
1	7.2	14.7	42.9
2	11.0	18.7	47.3
3	12.7	20.9	47.3

[1]The trees were classed as good condition before the first heavy defoliation.
Source: Adapted from Campbell and Valentine 1972.

Table 5–26.—Percent cumulative mortality among mixed oaks in fair and poor condition after heavy defoliation for 1, 2, or 3 successive years

Years after last heavy defoliation	Percent cumulative mortality, by tree condition, after number of years of heavy defoliation					
	1		2		3	
	Fair	Poor	Fair	Poor	Fair	Poor
1	17.1	33.3	42.0	42.7	55.0	56.2
2	26.2	43.5	51.8	59.9	60.6	67.1
3	28.8	47.9	57.2	59.9	60.6	67.1

Source: Adapted from Campbell and Valentine 1972.

foliation was one of the most significant variables related to subsequent mortality (Gansner et al. 1978).

Site Factors

The presence of dominant and codominant trees in poor condition before gypsy moth defoliation is a strong indication that other adverse environmental factors (stresses) have affected the stand. Drought, frost, and other defoliators, singly or in combination, have been implicated. Of course, these factors are frequently site related.

The tables summarizing the Melrose Highland data (Campbell and Valentine 1972) and the further treatment of these data by Campbell and Sloan (1977) provide an excellent account of the effects of different defoliation intensities on trees of different species, different sizes, and different crown conditions. In general, mortality increases with the number of years of heavy defoliation and is highest in small, suppressed understory trees and in trees in poor condition.

Defoliation, even when it is heavy, often goes unnoticed until it is very severe and covers large areas. High mortality rates attributed to a single year of heavy defoliation are suspect and suggest that earlier, undetected defoliations may have occurred. For example, in 1957, an outbreak of gypsy moth on Yeager Mountain near White Haven, Pa., completely defoliated an area of 12 ha. The following year, oak mortality was sufficiently heavy to affect stand composition materially. It is likely that building populations of gypsy moth had severely defoliated many trees for several years before the outbreak was observed (Nichols 1961).

Other factors may also account for an apparent excessive mortality rate following defoliation. Chief among these appears to be the condition of the trees at the time they were defoliated.

Stands growing on wet sites may suffer more seriously from lowered water tables than stands growing on normally dry sands or rocky ridges; frosts often affect stands on good growing sites in valleys and on lower slopes. Mortality can be high when frosts and insects defoliate the same trees twice in 1 year, or when severe drought periods precede or coincide with periods of heavy defoliation.

The early New England outbreaks occurred on a variety of soils and sites, but over time, repeated heavy defoliation has been most frequent on sites that are excessively drained and continually disturbed. In earlier discussions these stands were classed as highly susceptible. The questions frequently raised include: Are highly susceptible sites also high mortality sites and do trees on poor sites suffer more from gypsy moth defoliation than trees on good sites?

Recent studies seem to indicate that given the same defoliation regimen and no added, unusually severe environmental stress, small, slow-growing trees on excessively drained sites (deep sands or rocky ridges) are no more likely to succumb to gypsy moth than large, fast-growing trees on well-drained good sites. Indeed, the reverse appears true in many cases.

Some typically susceptible forests, such as on the Shawangunk Mountains in eastern New York, have a long history of heavy defoliation. Yet mortality there is not greater—often it is considerably lower—than on good growing sites after but 2 years of defoliation. High mortality can and does occur on the poor, dry sites. As mentioned earlier, however, these adverse sites are also the most likely to be defoliated repeatedly, and conditions on poor sites are often conducive to maintaining relatively high populations of mortality-causing agents such as the twolined chestnut borer. Severe protracted droughts also can compound the situation on excessively drained sites.

Trees growing on poorly drained sites (wet bottoms and benches with perched water tables) may suffer high mortality after defoliation. In fact, the highest mortality encountered in a current study of stand susceptibility was in wet-bottom stands. The reasons for high mortality in poorly drained sites are complex and probably include the facts that trees in low-lying sites are often subject to late spring frosts, are apt to suffer drastically from relatively slight drops in water tables, and often support tree species that seem to be especially vulnerable to defoliation. Indeed, species

composition is one of the most important factors associated with mortality following defoliation.

Species Composition

It has been known for a long time that some tree species are more vulnerable than others to defoliation. The Melrose data show that the magnitude of losses following repeated heavy defoliation was related directly to the percentage of oak in the stand (Campbell and Sloan 1977). Many workers have also shown that within the oaks themselves, the more highly preferred white oaks (*Q. alba* and *Q. prinus*) often suffer more than do members of the red-oak group (for example see Kegg 1973). Although there does seem to be a generally greater mortality in the white oak group, species mortality patterns following defoliation can be quite variable. For example, in the Morristown National Park in New Jersey, mortality after 2 to 3 years of heavy defoliation was 39, 38, 36, 16, and 11 percent for scarlet, white, red, black, and chestnut oaks, respectively (Kegg 1971). Yet on the Newark, N.J., watershed, similar levels of defoliation for the same time period resulted in mortality rates for white oak (88 percent) and chestnut oak (66 percent) that were markedly higher than for black, red, and scarlet oaks (47, 41, and 27 percent, respectively) (Kegg 1973).

The apparent greater vulnerability of the white oaks compared to the red oak species group probably stems partly from the fact that they are more preferred. (The word "preferred" is used advisedly. Common usage seems based on equating "preferred" with observed severe and often apparently differential defoliation of the white oak, not with actual feeding preferences. Recent unpublished studies suggest that white oak foliage may be less preferred than red-oak foliage, at least during the gypsy moth's first three instars. The higher apparent susceptibility of white oak may result from its other attributes, including its relatively high numbers of protective spatial niches, which enhance survival of older, more voracious instars.) At low gypsy moth population levels in mixed stands, the most preferred species are often the only ones defoliated (Campbell and Sloan 1977), and

it is highly probable, therefore, that differential, undetected defoliations of white oaks may occur for several years preceeding outbreaks. Even in outbreaks, white oaks may be defoliated to a higher degree than red oaks (Côté 1976).

Other possible reasons for higher vulnerability may be a greater rate of attack by *A. bilineatus* on defoliated white oaks than on other oak species (Côté 1976) and in inherent phenological and anatomical differences between the two oak groups. Budbreak in white oaks occurs 1 to several weeks later than in red oaks. Young white oak leaves are sometimes consumed as they expand by gypsy moths. While not yet studied, the effects of such an ill-timed encounter on a tree's energy reserves (lower to start with in white oak) could be highly significant, expecially for trees previously defoliated.

A similar effect can result when white oak is doubly defoliated in the same season by a combination of frost and gypsy moths. White oak occasionally suffers from late spring-frost damage when red oak does not—partly because of its phenology and partly because of its tendency in some areas to occupy low-lying valleys or benches at the foot of slopes. This vulnerability has figured prominently in recent attempts to develop models to predict mortality following gypsy moth defoliation.

Modeling Stand Mortality: Risk Rating

The many complex and interrelated factors contributing to tree mortality make it difficult to develop reliable models for predicting stand vulnerability. Nearly all models thus far produced are retrospective—that is, they attempt to predict what will happen in the future on the basis of specific cases of what has happened in the past. Examples of such models are the tables (Campbell and Valentine 1972) and mortality rate curves (Campbell and Sloan 1977) constructed from the Melrose case history data, the regression equations developed by Kegg (1974) for the tree mortality after two heavy gypsy moth defoliations of the Newark Watershed in north central New Jersey, and by Gansner et al. (1978) for tree mortality

that followed severe defoliations in eastern Pennsylvania.

In Kegg's (1974) regression models, the major independent variables were the percentage white oak group present (stems and basal area), aspect, percent exposed rock, and a stand susceptibility index (a summary variable comprised of relative density, relative basal area, and relative mortality by species).

In the Gansner et al. (1978) model, the two independent variables were the percentage white oak group in the stand and the condition of the tree crowns before defoliation. The importance of the percentage white oak group variable is obvious because these trees were important components of the stands and they suffered higher mortality rates than did other species. Possible limitations to these models, therefore, occur because white oaks are not always present in abundance in some highly susceptible stands and their mortality rates are not always excessively high following defoliation. White oak itself is a widespread and highly variable species. It is unlikely that its response to defoliation on the droughty soils of New England or even of New Jersey or Pennsylvania will be the same as that on deep loam soils or river bottom valleys of Ohio or Indiana.

Another approach now being explored is the use of principal components analysis ordination models to classify stands (Houston and Valentine 1977). The ordination patterns in figures 5–27, 5–28, 5–42, and 5–43 were obtained when forest stands were compared on the basis of their live tree structural features, which were ranked by the gypsy moth food-preference class of the tree species on which they occurred. The positions of those stands with oak mortality greater than 30 percent are identified. The patterns reveal two points of interest. First, the stands with high oak mortality are confined to relatively small portions of the models, and second, these stands are located primarily in areas of the ordination models where stands are classified as resistant. The first point suggests that it may be possible to use the models to predict stands where mortality will be high. The second point, which is more difficult to interpret, throws some caution on the first. Because only the

residual, living trees were used to produce the ordination patterns, the susceptible/resistant classifications pertain to extant stands. Death of susceptible oaks would have helped render stands more resistant. This is in agreement with the observations of other workers (Bess et al. 1947, Campbell and Sloan 1977, Kegg 1971, Nichols 1961, Stephens and Hill 1971).

On the other hand, other observations (Stephens and Hill 1971, Campbell and Sloan 1977 (citing personal communication from J.O. Nichols)) indicate that defoliation of forests on good growth (resistant) sites can result in heavy losses of fast-growing, dominant trees in good condition. This situation was the case for many of the stands in the ordination models with high mortality (Houston and Valentine 1977).

None of these models or approaches has been tested. Only with adequate testing under a wide range of field situations can their validity be assessed or their accuracy improved.
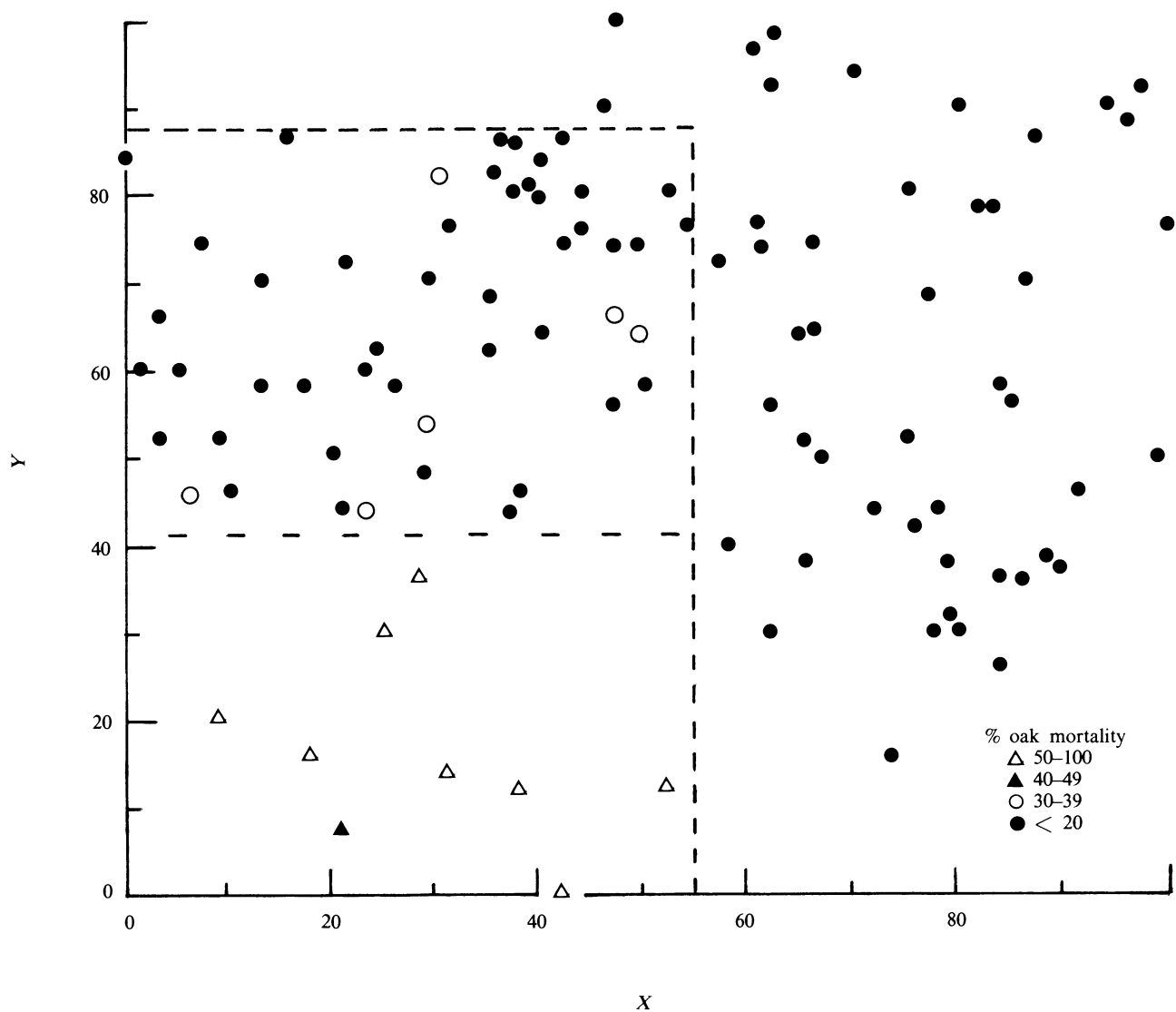
Figure 5-42.—*The* x–y *plane of a PCA ordination of 105 stands based on six tree structural features ranked by tree species separated into five gypsy moth food preference classes. The stand locations are identified by percentage oak mortality. The lines in this figure and in figure 5–43 delineate portions of the ordinations where stands incurred high oak mortality after defoliation.*
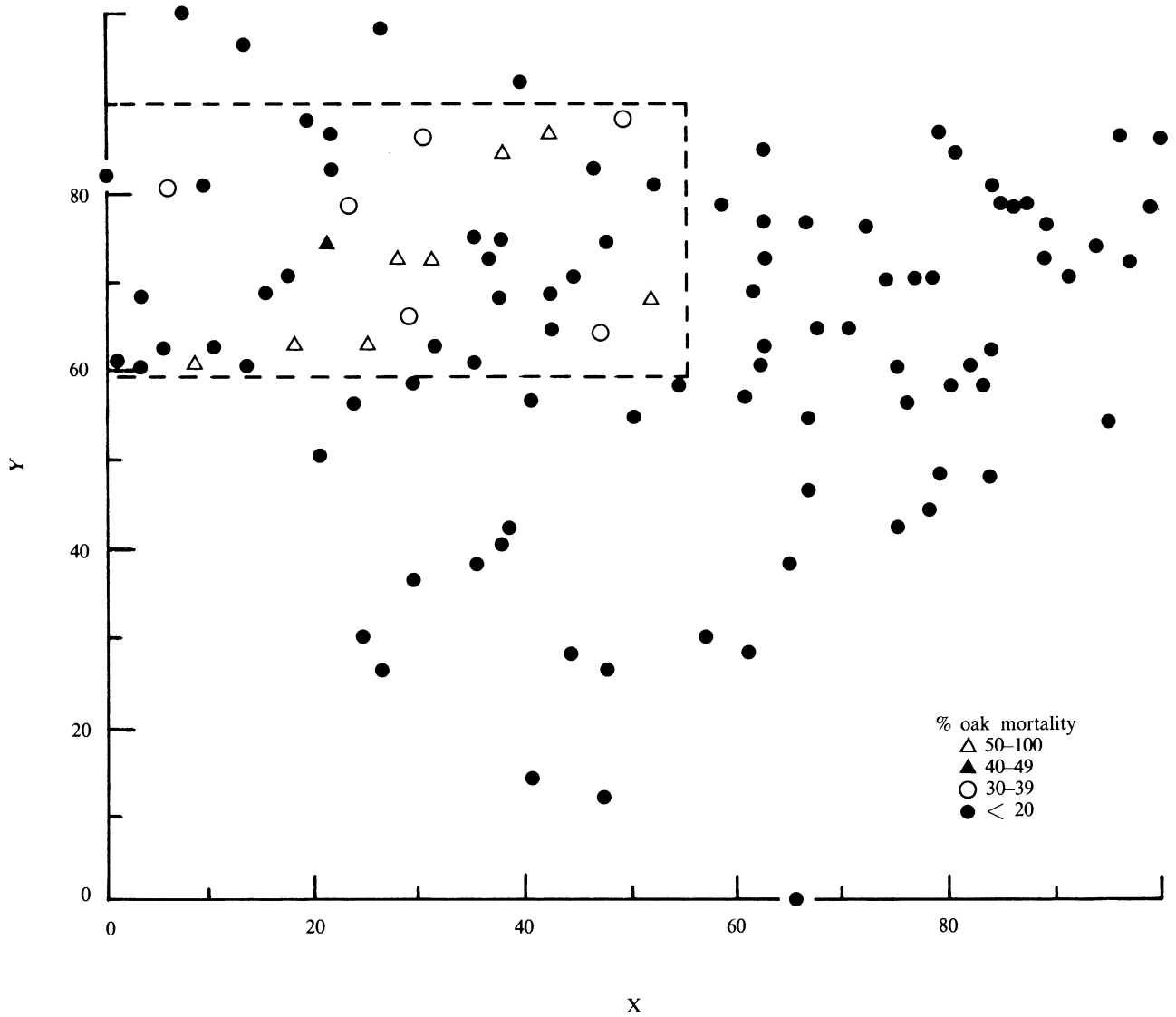
Figure 5-43.— *The* x-z *plane of the same ordination as figure 5-42.*

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Alternative Controls

6

6.1 Parasites

Introduction
Richard C. Reardon

Biological Control

Definitions

The characteristic abundance of a species is accomplished by the process of *natural control*, often described as "balance of nature," which is defined as the maintenance of population numbers (or biomass) within certain upper and lower limits by the action of the whole environment (Huffaker et al. 1971). The most important factors in natural control are natural enemies, weather and other physical factors, food (quantity and quality), interspecific competition (other than natural enemies), and intraspecific competition. The regulation by *natural enemies* of another organism's population density at a lower average than would otherwise occur is called *biological control* (DeBach 1974). Therefore, biological control rests on the premise that natural enemies are able to maintain the hosts' populations at lower levels than would occur in their absence. Biological control can be naturally occurring (by indigenous natural enemies), applied (manipulation of natural enemies by man), and fortuitous (accidental introduction of natural enemies).

All natural enemies are either parasites, predators, or pathogens and can occur in a great variety of forms: Vertebrates, arthropods, nematodes, snails and other invertebrates, microorganisms (pathogens that affect invertebrates, plant pathogens, and microbial antagonists), and higher plants (U.S. Department of Agriculture 1978). Only insect and nematode parasites are considered in this section. Parasitism is the association between two species in which one (the parasite) obtains its nutritional requirements from the body material of the other (the host); the host receives no benefits from the association and is not usually destroyed (Askew 1971). Parasites are distinguished from predators (which consume more than one individual in order to reach the adult stage) on the basis that the immature stages of parasites develop at the expense of a single individual.

Entomophagous insects (insects that feed upon other insects) include species that range from occasional feeders to obligatory parasites. In *obligatory parasitism* the attacking organism is limited to a parasitic existence; in *facultative parasitism* the attacking organism can upon occasion adapt itself to either a parasitic or a free life but ordinarily is not parasitic (Sweetman 1963). Insect parasites are considered to be different from true parasites in ways sufficient to set them apart and to justify the use of the distinguishing term *parasitoid*. They are recognized as being different because: The development of an individual destroys its host; the host is usually of the same taxonomic class; in comparison with their hosts they are of relatively large size; they are parasitic as larvae only, the adults being free-living forms; and they do not exhibit *heteroecism* (living as a parasite on first one host and then another) (Doutt 1964). In this section, however, the more inclusive term *parasite* will be used.

Five orders of insects contain species known to be parasitic upon insects: Strepsiptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera. The last two orders contain species that are of great importance in the biological control of pest insects. The Hymenoptera are the dominant order both in numbers of parasitic species and in the frequency and effectiveness with which they attack insect pests. The most important superfamilies of parasitic habit are the Ichneumonoidea (Ichneumonidae and Braconidae) and Chalcidoidea (many families). In the order Diptera, the Tachinidae are by far the most beneficial parasitic family of flies (Clausen 1972).

If a parasite attacks a nonparasite host, then the species is termed a *primary* parasite. If in turn the primary parasite is itself attacked, then its enemies are

called *secondary* parasites. Any degree of parasitism beyond primary is termed *hyperparasitism* (parasite feeds on the parasite and not the original host) and should not be confused with *cleptoparasitism* (parasite destroys the parasite and feeds on the original host). A *solitary* parasite feeds and develops as a single parasite in or on the host, whereas a gregarious parasite feeds and develops on the host along with one or more members of the same species. Two additional categories of parasitism are *superparasitism* (parasitization of an individual host by more larvae of a single parasitic species than can mature in that host) and *multiple parasitism* (simultaneous parasitization of a single individual host by two or more different species of primary parasites).

Efficiency of a Parasite

Huffaker et al. (1971) list characteristics that are presumably pertinent to the efficiency of a parasite: Fitness and adaptability to various physical conditions of the environment; searching capacity; rate of increase relative to that of the host; synchronization with host; host specificity; ability to survive host-free periods; and special behavioral traits that alter its performance as related to density or dispersion of its host and its own population. Successful biological control following release of parasites generally requires time for three or more generations of reproductive increase (numerical response) of the parasite.

Applied Biological Control

Augmentation and Conservation

Applied biological control implies manipulation of natural enemies by man. DeBach and Hagen (1964) divided the management of parasites into two categories: Augmentation, or manipulation of the parasite itself, and conservation, or manipulation of the environment to favor the parasite. The augmentation of parasites to increase their effectiveness involves their direct manipulation either by mass production and periodic colonization or by planned genetic improvement. The term *colonization,* in biological control,

refers to the attempted establishment of an organism (parasite) in a new locality and implies knowledge of the biological and ecological interrelationships of the parasite and its hosts (DeBach and Bartlett 1973).

Classical Control

The discovery, importation, release, and establishment of exotic species of parasites are all part of *classical biological control*. Although this approach has proven the most successful, DeBach (1974) comments that much still is to be done, because between 70 and 90 percent of all parasitic Hymenoptera are yet to be discovered. Coppel and Mertins (1977) list two situations where the introduction of exotic natural enemies are appropriate: If there exists a vacant niche (or niches) in the life system of the pest that might be filled by the introduced species, or if the present occupant of a niche is an inherently inefficient regulator of the pest and is susceptible to displacement by a more efficient introduced regulator. Classical biological control encompasses several opposing philosophies: single species introduction (the one species most promising) versus multiple (two or more species, although sometimes more precisely defined in terms of the same stage of development of the host, to distinguish from sequential, several parasite species that each parasitize a different stage of the host); polyphagous (multitude of hosts) versus oligophagous (very few and often closely related hosts) or monophagous (specific to one particular host) species; suitablility of natural enemies from closely related hosts; and influence of hyperparasites. In general, the introduction of an additional parasite has maintained or improved upon the degree of overall control, while most of the outstanding successes have been obtained from highly host-specific parasites. An example of successful biological control using introduced parasites of a forest-defoliating species is the winter moth, *Operophtera brumata* (L.), in eastern Canada (Embree 1966).

Both the augmentative and classical approaches of using parasites have been used in an attempt to suppress populations of the gypsy moth, *Lymantria dispar* (L.), in the Northeastern United States. In fact, the

foreign exploration and importation of exotic species of parasites of the gypsy moth have been part of one of the most massive programs in biological control history (Brown 1961).

Foreign Explorations

Past History: 1900–60
Richard C. Reardon

Gypsy Moth Parasite Establishment in North America

The gypsy moth was introduced into Massachusetts from France in 1868 or 1869, and for approximately 10 years it was found only in the immediate vicinity of the release site (Forbush and Fernald 1896). It did not come into public notice until the summer of 1889, and from 1890 to 1900 attempts were made to eradicate this insect (Burgess and Baker 1938). During this time, several entomologists commented that natural enemies were not effective in preventing the spread of the gypsy moth, even though it was generally held in check by natural enemies in Europe (Howard and Fiske 1911). In 1891, C. V. Riley suggested sending one or two persons to northern Europe to collect and transmit parasites of the gypsy moth. Although no one was sent to Europe, earlier in the fall of 1890 the U.S. Department of Agriculture received from Rev. H. Loomis of Yokohama, Japan, a letter expressing interest in the gypsy moth infestation in Massachusetts. He also forwarded parasites, later identified as *Apanteles liparidis*, which he had observed parasitizing gypsy moth larvae in Japan; unfortunately all arrived dead. C. H. Fernald commented in 1896 that no attempt had been made to import parasites because the law required that suppressive efforts be directed toward extermination of the gypsy moth.

Defoliation by the gypsy moth was minimal in 1898 and 1899; by February 1900, only 92,981 ha in the immediate vicinity of Boston were infested by the gypsy moth. This "low profile" brought about an abandonment of the effort to eradicate the gypsy moth. Subsequently, within 4 years the earlier infested areas had become reinfested and the insect had spread widely (576,037 ha infested in Massachusetts, and many localized infestations were found in Maine, New Hampshire, and Rhode Island), thereby renewing interest in importing exotic species of parasites to suppress the gypsy moth and slow its further spread.

Foreign Exploration for Exotic Species of Parasites

In 1904, the State of Massachusetts and the Federal Bureau of Entomology appropriated funds and agreed to cooperate in importation of natural enemies of the gypsy moth. Representatives from both agencies decided to confine the first efforts at obtaining parasites to European countries, because the Massachusetts infestation had developed from eggs brought from Europe. This was in recognition of the differences between the European and Japanese forms and because of the problems involved with long-distance transport of parasites from Japan (Summers 1924). The plan was to secure as many parasites belonging to as many different species as possible from all parts of Europe. The simplest way of securing the parasites was to collect the various host stages in Europe and transport them to a laboratory established in Massachusetts (Howard and Fiske 1911).

In 1905, L. O. Howard (assigned by the U.S. Department of Agriculture to supervise the study and introduction of natural enemies) sailed to Europe to plan for the collection, handling, and shipment of parasites to the United States. Between 1905 and 1910, host material was collected in Europe, Japan, and Russia and forwarded to the Massachusetts laboratory. By the end of the 1910 season, it was generally accepted that a sufficient number of species had been secured and that it would be more useful to study in the countries of origin those species of apparent importance that had not become established after importation (Howard and Fiske 1911).

In 1905, small shipments of parasites had been unsuccessfully shipped from Japan. In 1908, T. Kincaid of the University of Washington was sent to Japan and shipped a number of species of parasites in the

next 2 years. After 1910, parasite investigations were confined to Europe until the outbreak of World War I in 1914, which put an end to all such activities.

Parasite importation was reinstated in 1922 by the Federal Bureau of Entomology, with S. S. Crossman searching for gypsy moth infestations in France, Spain, Italy, and Germany. Except in Spain, he was unable to locate an infestation of sufficient size to make large collections of larvae. In March 1923, S. S. Crossman and R. T. Webber made a search for gypsy moth infestations in France, Spain, Italy, Germany, Austria, Hungary, Rumania, and Poland. Only in two of these countries (Hungary and Spain) were gypsy moth infestations large enough to warrant the establishment of temporary laboratories for gypsy moth collection (Burgess and Crossman 1929). In Hungary, for example, approximately 115,000 gypsy moth larvae and pupae were collected, from which 43,873 parasites were recovered (Crossman and Webber 1924).

In 1922 and 1923, J. N. Summers coordinated the search for parasites in Japan; at the close of the 1923 season he felt that the work in Japan should be discontinued in favor of the more promising field in Europe (Summers 1924).

The search for parasites of the gypsy moth continued in Europe until 1933. The parasites received at the Massachusetts gypsy moth laboratory during these years were obtained, for the most part, by rearing them in the native countries from immature gypsy moths. The purpose of this foreign work (1922–33) was to introduce several natural enemies of the gypsy moth that apparently had not become established during former importation work, because either they were never received and liberated in satisfactory numbers or because they were liberated under conditions not favorable to their establishment (Burgess and Crossman 1929).

During both periods of foreign exploration, 1905–14 and 1922–33, host and parasite collections were generally made from high-density gypsy moth populations. Limited foreign exploration was not resumed until the 1960's.

Recent History: 1961–77
Jack R. Coulson

Introduction

In the early 1960's, following the halt of large-scale aerial application of DDT for control of the gypsy moth, a number of alternate methods were sought for the control of this pest, which seemed on the verge of rapidly expanding its range in the Northeastern United States. The exploration for and introduction of additional exotic parasites and predators of the gypsy moth or of closely related species were early considerations (Brown 1961, Leonard 1974). Efforts were resumed in 1960 to search the world for new natural enemies, the addition of which to the complex already established might assist in maintaining gypsy moth populations at more tolerable levels in the United States.

Early Overseas Studies Sponsored by the Forest Service

In the late 1950's, the Special Foreign Currency Research Program under Public Law 480 was initiated, the authority for which is the Agricultural Trade Development and Assistance Act of 1954, as amended. Under this program, the USDA uses excess currencies in various countries, resulting primarily from the sale of agricultural commodities to those countries by the United States, to support agricultural and forestry research on problems of mutual interest to the United States and the participating countries. The utilization of these funds to support biological control studies in foreign countries was recognized to be most appropriate.

There have been five Public Law 480 projects concerning studies of the gypsy moth and its natural enemies (and several relating to pathogens of the gypsy moth) supported by the Forest Service: A 5-year project in Spain, 1960–65; two consecutive projects in India, 1961–72; and two consecutive projects in Yugoslavia, 1967–75. In addition to the

accumulation of much basic information on natural enemies of the gypsy moth in these three widely separated areas of the world, a number of the natural enemies studied under the projects were imported for study and release in the United States.

Early Overseas Studies Sponsored by the Plant Protection Division and the New Jersey Department of Agriculture

In 1967–69, Plant Protection Division (PPD, then part of Agricultural Research Service, ARS, and now the Plant Protection and Quarantine Programs of the Animal and Plant Health Inspection Service) personnel in Spain (in connection with the division's studies on sex attractants) brought back large quantities of parasites into quarantine for use in culture and release programs. In 1970, the New Jersey Department of Agriculture (NJDA) funded a graduate student's travel and studies of the gypsy moth in Yugoslavia, which also resulted in the shipment of considerable numbers of natural enemies to the United States.

Overseas Exploration by the Agricultural Research Service, 1972–77

From 1963–71, ARS involvement in the gypsy moth natural enemy importation program was only in the quarantine receipt and clearance of foreign natural enemy material obtained in the overseas studies and collections for further shipment to PPD and NJDA. In 1972, funds were made available to ARS to carry out explorations and studies of gypsy moth natural enemies. This came about for three reasons: The increasing demand by various State agencies for more foreign explorations and for more exotic species of natural enemies, the possibility of discovery of hitherto unknown gypsy moth natural enemies in areas of the world not completely explored during earlier overseas collection activities and the establishment of previously discovered but unestablished species.

Two large areas, the Soviet Union and People's Republic of China, unfortunately remained inaccessi-ble to ARS exploration teams. In Europe, however, where studies had emphasized collections and studies in high host-population densities, new studies were designed to concentrate as much as possible on low host-population densities. It was hoped that new parasite species effective in low densities of the gypsy moth might be found.

As for the establishment of previously discovered species, two factors were involved. First, it was believed that the increased number and kinds of ecological niches available throughout the now-expanded range of the gypsy moth might lead to establishment of some. of the natural enemies discovered and introduced but unestablished in earlier release programs. Second, more rapid transportation facilities and increased knowledge in handling and culture techniques might increase the possibility of establishing species that had been released in inadequate numbers during the earlier programs.

In 1972, a gypsy moth project was established at the ARS European Parasite Laboratory in France, to recommence exploration and collection work in Europe. In addition, domestic field studies of the efficacy of established populations of gypsy moth natural enemies were initiated at the ARS Beneficial Insects Research Laboratory in New Jersey (relocated in 1973 to Newark, Del.). During 1972–73, the ARS studies in Europe were concentrated on low host-population densities in southeastern France. In 1974, similar studies were made in eastern Austria, with some additional collections provided by collaborators in Germany and Corsica.

In 1975, with funds made available as a result of the newly initiated Combined Forest Pest Research and Development Program (Ketcham and Shea 1977), ARS increased its efforts in Europe, with the addition of a designated gypsy moth team there, and at its Newark, Del., quarantine laboratory. In May 1975, ARS was able to initiate studies in Japan and Korea with the opening of the ARS Asian Parasite Laboratory at Sapporo, Japan. Thus, in 1975, studies in Austria were completed, and explorations and studies were also conducted in central France and

central Poland, as well as in Japan and Korea. Attention then focused on the Orient, where explorations are continuing. Explorations were conducted in Iran in 1976; in 1977 a European team was sent to assist with the exploration and collection work in Japan.

Mention must be made of the invaluable assistance provided to these programs by the ARS Systematic Entomology Laboratory (SEL) located in Washington, D.C., and Beltsville, Md. Taxonomists of SEL provided the majority of identifications of host and natural enemy species collected during the course of ARS and other overseas explorations, as well as those studied in most Federal and State domestic programs. SEL, in cooperation with the Forest Service, provided a recently published detailed study of the tachinid parasites of the gypsy moth throughout the world; the study includes up-to-date information on current nomenclature, distribution, host ranges and biologies, a description and key to the adults and puparia, and notes on introduction to the United States (Sabrosky and Reardon 1976). A similar work by SEL on the braconid parasites of the gypsy moth is nearing completion.

Other Overseas Studies Sponsored by the Agricultural Research Service

In addition to the exploration and studies performed at the two ARS overseas laboratories, ARS sponsored additional overseas studies under the Public Law 480 program or by direct contract beginning in 1972.

In 1972, ARS contracted with the European station of the Commonwealth Institute of Biological Control (CIBC) for a survey of Japan as a potential area for further search for gypsy moth natural enemies. In the same year, ARS entered into an exchange agreement with the Soviet Union, one aspect of which included an exchange of biological control agents between the two countries; natural enemies of the gypsy moth were included among the material requested by the United States. Only a single shipment has been received from each country as a result of these agreements.

Two Public Law 480 3-year projects for surveys and collections of gypsy moth natural enemies were also negotiated by ARS in 1972 in Yugoslavia and Morocco. In 1975 through 1977, ARS entered into contracts with the CIBC Station at Bangalore, India, for additional collection of specific natural enemies identified during earlier studies in India and requested by several U.S. research workers.

Exotic Natural Enemies Received in the United States, 1963–77

A record of the species of natural enemies imported from 1963 through 1977 is presented in table 6.1–1. It is important to note that a number of the species listed in this table have not been recovered from the gypsy moth, L. dispar, as it is known in North America and Europe. All natural enemies received from India have been collected from the "Indian gypsy moth," L. obfuscata (Walker). The "Japanese gypsy moth" currently is being treated as a subspecies, L. dispar japonica (Motschulsky) (Ferguson 1978). The taxonomy of the "gypsy moths" in Japan and Korea remains in a state of confusion; Goldschmidt (1934, 1940) reported the existence of extreme variability of populations in Japan, and several "species" or "subspecies" are reported from these areas. This variability and other differences in Japanese populations are discussed briefly in the section on the ARS Asian Parasite Laboratory. Several of the species listed in table 6.1–1 were collected from hosts closely associated with gypsy moth infestations in foreign areas for trial on L. dispar in the United States (see footnotes 3, 19, and 20 of table 6.1–1).

Problems with specific identification of some of the exotic material received prevented an exact accounting of the total number of species received, thus the use of the ± symbol when discussing the number of species imported. Specific identification was not always possible, especially if voucher specimens were not kept. Material is often listed with only generic determination and may include species already listed or new or unidentified species only rarely associated with the gypsy moth. In addition, several determina-

Table 6.1-1—*Foreign species of gypsy moth natural enemies shipped to the United States, 1963-77*

Taxonomic classification and genus and species	Origin of collections and years of shipment[1]			Shipped from quarantine[2]
	1963-71	1972-74	1975-77	
Hymenoptera				
Braconidae				
Apanteles lacteicolor Viereck			F[3], In, Ir	+
A. liparidis (Bouché)	In[4], S?[5], Y[6]	A, F, G, J, Y	A, F, In, Ir, J, M[7], P, Y	+
A. melanoscelus (Ratzeburg (=*A. solitarius* (Ratzeburg))	In[4], Y[6]	A, F, G, M, Y	A, F, Ir, J, P	+
A. porthetriae Muesebeck	S[5]	A, F, G, M	A, C, F, M[7], P	+
A. ?ruidus Wilkinson			In	+
A. sp. "nr. *conspersae*"	In?[4]			?
A. n.sp.			J	+
A. spp. indet.	In?[4], S?[5], Y?[6]	In*	In*	?
Meteorus pulchricornis (Wesmael)		A, F, M, Y	A, Ir, M, P	+
M. versicolor (Wesmael)[3]			F	+
M. spp. indet. (2 spp.)		F	J*	+
Rogas indiscretus Reardon (=*Rogas* sp.)	In[8]		In	+
Rogas lymantriae Watanabe			J	+
Ichneumonidae				
Casinaria tenuiventris (Gravenhorst)		A, F, G		+
C. spp. indet. (2 spp.?)		F	A*	+
Coccygomimus disparis (Viereck) (=*Pimpla* sp.; *Coccygomimus* sp.)		In[9]	J	+
C. instigator (F.)		M, Y	Ir, P	+
C. turionellae turionellae (L.) (=*Pimpla* sp.)		In		+
C. turionellae moraguesi (Schmiedeknecht) (=*Pimpla* sp.)		M		+
C. spp. indet. (=*Pimpla* sp.)	In*[10]	C	M*	+
Hyposoter tricoloripes (Viereck)		A, F, G	A, F, P	+
Ichneumonid spp. indet.	Y	M	M*	+
Lymantrichneumon disparis (Poda)			Ir	-
Meloboris (s. lat.) sp.[3][11]			P	+
Phobocampe disparis (Viereck)		A, F, G, Y	A, F, P	+
P. spp. indet.	Y	A*, F*, G*, Y*	Ir*, J*, P[12]	+
Tranosema rostrale (Brischke[3][11]) (="*T.* arenicola")			P	+
Vulgichneumon sp. indet.[13] (="*Melanichneumon* sp.")		M*	M	+
Eulophidae: *Euplectromorpha laeviuscula* (Thompson)		F		-

Table 6.1-1—Cont.

Taxonomic classification and genus and species	Origin of collections and years of shipment[1]			Shipped from quarantine[2]
	1963–71	1972–74	1975–77	
Encyrtidae: *Ooencyrtus kuvanae* (Howard)		F, M*[14]		+
Eupelmidae:				
Anastatus disparis Ruschka		F	A, F	+
A. ?kashmirensis Mathur			In	+
A. spp. indet.	In[15]	F[16]	Ir*	+
Torymidae: *Monodontomerus* aereus Walker[17]	In			–
Chalcididae:				
Brachymeria "*euploeae* (Westwood)"			In	+
B. intermedia (Nees)	In, S	C, F, M, Y	In*, M*	+
B. lasus (Walker)			J	+
B. spp. indet.		In[18]	In*[18]	+
Scelionidae:				
Gryon sp. indet.[19]			M	–
Hadronotus sp. indet.		F		–
Telenomus spp. indet. (2–3 spp.?)		F	M[19]	–
Family indet.: "Hymenoptera sp. indet."	Y			+
Diptera:				
Sarcophagidae: *Agria affinis* (Fallén)		F, Y		+
Tachinidae:				
Blepharipa pratensis (Meigen) (=*B. scutellata*)		A, C, F, G, Y	A*, F*, P *	+
B. schineri (Mesnil)[20]			J	–
B. "*sericariae* (Rondani)"[3]			J	–
B. sp. indet.			J	–
"*B.* sp." indet.[20]			J	–
Blondelia nigripes (Fallen)		A	P	+
Carcelia separata (Rondani) (= "*C. excisa*")		A, F	A, Ir, M, P[21]	+
C. sp. indet.[22]		Y		+
Compsilura concinnata (Meigen)		A, F, Y	Ir*, J*, M*, P*[21]	+
Euphorocera sp. indet.		F		+
Eusisyropa sp. indet.		F		+
Exorista japonica (Townsend)			J	+
E. larvarum (L.)	Y[23]		Ir	+
E. rossica Mesnil	In	In		+
E. segregata (Rondani) (=*Tricholyga segregata*)	S			+
E. sorbillans (Wiedemann)[3]			K	–
Palexorista disparis Sabrosky[24] (="*Drino discreta*"; *Palexorista* sp.)	In	In		+
P. inconspicua (Meigen) (="*Drino inconspicuoides*")	In	F		+
P. spp. indet. (2 spp.?)		F, Y	M*	+
Parasetigena silvestris (Robineau-Desvoidy) (=*P. agilis*)		A, F, G, Y	A*, F*, J*[20], P*[21]	+

Table 6.1-1—Cont.

Taxonomic classification and genus and species	Origin of collections and years of shipment[1]			Shipped from quarantine[2]
	1963-71	1972-74	1975-77	
P. sp. indet.			J	–
"P. sp." indet.[3]			K	–
Phorocera assimilis (Fallén)[20]			J	–
"P. sp." indet.		Y		+
Siphona samarensis (Villeneuve)		A		–
"Sturmia sp." indet.	Y			–
Tachinid spp. indet. (3-4 spp.?)[25]	Y	Y*, M*	Ir*[26], J*, K*, Y*	+
Hemiptera:				
Pentatomidae:				
Dinorhynchus dybowskyi Jakovlev			J	–
Pentatomid sp. indet.			Ir	–
Coleoptera:				
Carabidae:				
Calosoma sycophanta (L.)		A, Y	A*, Ir*, M*	+
Dermestidae:				
Anthrenus verbasci (L.)		M		–
Trogoderma versicolor (Creutzer)		M		+
Trogositidae:				
Tenebroides maroccanus Reitter		M		–
Tenebrionidae:				–
Akis bacarozzo (Schrank)			M	–
Nematoda: Mermithidae: Hexamermis "albicans" (Siebold)"[27]		A, G	A, J, U	+
Pathogens: Viral, fungal		A, G	A, In, Ir, J, P	+

[1]A = Austria; C = Corsica; F = France; G = Germany; In = India; Ir = Iran; J = Japan; K = Korea; M = Morocco; P = Poland; S = Spain; U = USSR; Y = Yugoslavia.

[2]A plus sign (+) in this column indicates shipments of the species were made from quarantine. An asterisk (*) in the Origin columns indicates material from that source was not included among material shipped from quarantine.

[3]Collected from hosts other than gypsy moth for trial on the latter: Apanteles lacteicolor and Meteorus versicolor from France were recovered from the browntail moth, Nygmia phaeorrhoea (Donovan); Meloboris (s. lat.) sp. and Tranosema rostrale from Poland were recovered from the rusty tussock moth, Orgyia antiqua (L.); and Blepharipa "sericariae" from Japan and Exorista sorbillans and "Parasetigena spp." from Korea were recovered from the silk moth, Bombyx mori L.

[4]"Apanteles spp." shipped from India in 1970-71 were said to include A. melanoscelus, A. liparidis, "A. sp. nr. conspersae," and possibly other species.

[5]"Apanteles spp." shipped from Spain in 1964-65 and 1968-69 were first shipped as A. "vitripennis"; they were later found to include primarily A. porthetriae, but possibly also A. liparidis and other species.

[6]Apanteles spp. shipped from Yugoslavia in 1970 included primarily A. liparidis with some A. melanoscelus and possibly other species, including A. ocneriae Ivanov.

[7]Shipments of Apanteles spp. from Morocco in 1975 were a mixture of A. liparidis and A. porthetriae.

[8]Early shipments (1967-68, 1970) from India were called Rogas sp., prior to its formal description.

[9]This species, originally shipped from India in 1972 as "Pimpla sp." and cultured and released in the United States as "Coccygomimus sp.," has recently been identified as C. disparis by R. W. Carlson, USDA Systematic Entomology Laboratory.

[10]This material shipped from India in 1971 was never identified and may have been either C. disparis or C. turionellae turionellae.

[11]These species were included in 1975 shipments of "Hyposoter spp." from Poland.

[12]Included in a 1975 shipment of P. disparis from Poland.

[13]Included in shipments of "miscellaneous ichneumonids" from Morocco in 1974-75; other miscellaneous species are undetermined.

[14]The 1973 Moroccan material was originally identified as "O. obscurus."

[15]The Anastatus spp. from India in 1966-67 may have included A. ?kashmirensis and other species.

[16]The "Anastatus sp." from France in 1972 was called "blue" and was differentiated from A. disparis, but may have been the latter species.

[17]This species acts primarily as a hyperparasite on dipterous and hymenopterous species, although it also acts as a primary parasite of the gypsy moth and other lepidopterous species.

[18]The 1972 and 1976 material from India was probably B. intermedia but may have included B. "euploeae"; the few adults that emerged from the 1976 material were B. intermedia.

[19]Shipments of "Telenomus spp." from Morocco in 1975-76 included 1-2 species of Telenomus and a species of Gryon; the latter may possibly be a parasite of pentatomid eggs rather than of gypsy moth eggs.

[20]Collected as adults from a gypsy moth infestation in Japan, 1977; actual hosts unknown.

[21]Shipments from Poland in 1975 under these names included some Blondelia nigripes.

[22]This 1973 shipment from Yugoslavia was apparently never identified and may have been *C. separata.*

[23]This 1970 material from Yugoslavia was shipped among other "tachinid spp."

[24]Shipments of this species, as "*Drino discreta,*" may have included some *P. solennis* (Walker) (see Sabrosky and Reardon 1976).

[25]Many shipments of tachinid puparia were received from which no adults emerged and thus no identifications were made; these may represent species listed above.

[26]Included at least *Carcelia, Compsilura,* and *Exorista* as listed above and probably other species that remain unidentified.

[27]More than one species may be involved in this complex.

tions of 1976 and 1977 material are still pending. The major problem was to obtain identifiable adult specimens from the foreign material, which was usually received in immature stages. These stages are difficult or often impossible to determine to species and often even to genus. For example, heavy hyperparasitism of some incoming shipments, particularly of braconids, and the difficulty of adequate overwintering under artificial conditions of univoltine species, especially tachinid species, have resulted in a number of cases in which no adults at all were obtained for identification.

During the past 15 years, over 211,000 individuals of ± 78 species have been sent to the ARS quarantine station in the United States from collection areas ranging from Morocco and Western Europe to Iran and India, Korea, and Japan. However, the majority of these individuals represent only a few of the species listed in table 6.1-1. Many of the species listed were shipped in very small numbers because of the difficulty in their collection, and a few other species, not listed here, were collected during foreign explorations in too few numbers to allow shipment at all.

A total of ± 53 species (over 82,000 individuals) of exotic gypsy moth natural enemies were shipped from ARS quarantine. The majority of the individuals shipped from quarantine represent only a few of the species received. The smaller numbers of both species and individuals shipped *from* quarantine reflect the problems of hyperparasitism, inadequate overwintering, other mortality during shipment and quarantine activities, and, in later years (1975–77), some lack of interest of U.S. workers in receiving additional

specimens of some of the more common species previously received and studied.

Note that predators and pathogens received from foreign collections are included in table 6.1–1 for the sake of completeness. Of the former, only one species was shipped from quarantine.

Observations on Program Results

As a result of these overseas studies and collections, several general observations can be made.

a) In general, and particularly throughout Europe, Morocco, and Iran, the more effective gypsy moth natural enemy complexes were found to be quite similar wherever studies were conducted, with some local variations. This was also true, to a somewhat lesser extent, in India, where the gypsy moth, *L. obfuscata,* is not the same species as that of Europe and North America, although the two species are very closely related. Although many of the parasites found in India were the same as or close ecological homologues of those found in Europe, a few apparently unique species were found, notably *Rogas indiscretus.* This is also true in Japan and Korea, where the local gypsy moths may be subspecies of *dispar* or distinct species. Additonal biological and taxonomic studies of the gypsy moth populations and their natural enemies are required in Japan and Korea, but preliminary data indicated the existence of a natural enemy complex similar to that found in Europe, with some different ecological homologues and other apparently unique species.

b) Most of the over 211,000 individuals of natural enemies collected and shipped during 1963–77 consisted of the more prevalent species found. These were most often either species already established in the United States from previous introduction programs, or species that had been unsuccessfully introduced earlier and that are known or believed to require alternate hosts probably not present in the areas of release in the United States.

c) Many of the ± 78 species collected and shipped in small numbers during 1963–77 and several species

found in too small numbers to ship were known or later found to be either only incidental parasites of the gypsy moth or widely polyphagous species.

d) Very few new natural enemies of the gypsy moth were found during the recent overseas studies. Most of these were from Indian and Japanese gypsy moths, and few currently show any great potential for effectiveness against the gypsy moth in North America.

The objectives of the ARS and other exploration programs included: a study of the gypsy moth and its natural enemies in various geographical areas; the discovery and introduction of new species of gypsy moth natural enemies, and the introduction of species previously introduced but unestablished; and finally, establishment of these species to increase the pressures caused by natural enemies in order to lessen, either alone or in combination with other control measures, the intensity of damage by the gypsy moth to a more tolerable level.

The Search in Various Geographical Areas

Table 6.1-1 indicates that a large portion of the world has been explored, or in some cases re-explored: studies have been conducted in Europe (Spain, France, Corsica, Germany, Austria, Poland, and Yugoslavia), North Africa (Morocco), and Asia (Iran, India, Japan, and Korea). The U.S.S.R. and People's Republic of China are areas where exploration may be of benefit but where travel is currently difficult or impossible. Studies in Afghanistan and Pakistan may be warranted and could possibly be carried out. Exploration in other areas where the gypsy moth is known to occur—northwestern Europe, the Near East, Algeria, Tunisia, and Taiwan—is currently not warranted because natural enemy complexes are believed to be similar to nearby already explored areas. Furthermore, gypsy moth populations in these areas approach their geographical/climatic limits or represent comparatively recent invasions, with probably consequent depauperate natural enemy complexes.

The Discovery and Introduction of New or Previously Unestablished Parasite Species.

During the explorations, special efforts were made to discover: Egg parasites of more effectiveness than the two now established in the United States (*Ooencyrtus kuvanae* and *Anastatus disparis*), whose effectiveness is limited by the fact that they are capable of parasitizing only the outer layer of eggs of gypsy moth egg masses (some efforts were also made to discover egg predators capable of opening up egg masses for deeper penetration); parasites of small larvae to supplement parasitism of *Apanteles melanoscelus,* the only effective parasite of small larvae that is established, but whose effectiveness is limited by heavy hyperparasitism; and pupal parasites to supplement parasitism by the established *Brachymeria intermedia,* whose activity seems limited to areas of heavy defoliation. In addition to the introduction of largely polyphagous species, some of which are locally abundant in foreign areas (chiefly *Exorista* and *Palexorista* species), and a number of incidental parasites, some progress was made in the discovery of more promising parasites of eggs, small larvae, and pupae.

Egg parasites include *Telenomus* species from *L. dispar* and *Anastatus ?kashmirensis* from *L. obfuscata.* The former were received only in small numbers, and taxonomic difficulties prevented proper identification that might lead to their effective utilization. The Indian *Anastatus* has been shown in laboratory studies to be capable of facultative hyperparasitism of braconid cocoons (as are *O. kuvanae* and *A. disparis*), although no such hyperparasitism has been recorded in the field. Because of this potential facultative hyperparasitism, some researchers are reluctant to field release the species in the United States, and it has not been released. Moreover, current information concerning both *Telenomus* and *A. ?kashmirensis* gives no indication that either species would be more effective than the egg parasites already established.

Promising larval parasites include *Rogas indiscretus* from India, *R. lymantriae* from Japan, and several

species of *Apanteles* (a new species from Japan, the European *A. porthetriae,* and particularly the cosmopolitan *A. liparidis*). Concerted efforts to establish *R. indiscretus* have apparently been unsuccessful. More biological information is needed on this species and on the recently imported *R. lymantriae,* as well as on the Japanese *Apanteles* species. Considerable culture and release activities have been conducted with *A. liparidis* and *A. porthetriae* during recent and past exploration programs, including attempts to discover alternate hosts that would permit these multivoltine species to establish and overwinter in North America. To date these efforts have apparently been unsuccessful, and these two species remain unestablished.

Pupal parasites include several polyphagous *Coccygomimus* species and the more promising, recently imported *Brachymeria* species from India and Japan. Widescale culture and release efforts with the *Coccygomimus* species have apparently failed to result in their establishment. Additional biological information is required concerning the *Brachymeria* species, which, like, *B. intermedia,* may demonstrate some degree of facultative hyperparasitism of tachinid puparia in laboratory studies, although to what extent this might occur in nature is uncertain.

Three other new and promising natural enemies discovered in the recent explorations are the nematode parasite complex *Hexamermis "albicans"* from Europe and Japan; the pentatomid predator *Dinorhynchus dybowskyi;* and a fungus disease that was found to cause at least one epizootic collapse of a gypsy moth population in Japan.

Future Exploration Plans

Immediate ARS (now Science and Education Administration—Agricultural Research) plans include continued study in Japan and Korea of the gypsy moth and its natural enemies at least through 1978 and possibly 1979, to attempt to answer some of the many biological and taxonomic questions concerning gypsy moth populations there. Continued exploration of populations in India, and perhaps initiation of such studies in Pakistan and Afghanistan, depend on the

results of domestic studies currently being conducted with previously imported Indian species. The only study planned in Europe is a short exploratory study in Rumania in 1978, primarily for collection of several pupal parasites. Long-range plans include survey and collection studies in the U.S.S.R. and People's Republic of China, if current travel restrictions in these countries are eased.

Public Law 480 Projects and Other Miscellaneous Overseas Activities: 1960–77

Forest Service Sponsored Public Law 480 Project in Spain, 1960–65:
Richard C. Reardon

Project No. E25–FS–10, "The Study of Parasites, Predators and Diseases of the Gypsy Moth and the Possibility of their Application in the Biological Control," was conducted from May 11, 1960, to May 11, 1965. N. Romanyk, Servicio de Plagas Forestales, C. Marques de Mondejar 33, Madrid, Spain, was principal investigator of this project. The following is brief résumé of the final report of the project. Shipments of natural enemies resulting from the project are shown in table 6.1–1 in the Recent History section.

The gypsy moth in Spain is one of the major defoliators, causing principal damage in the oak forests with marked preference for the evergreen oak (*Quercus ilex* L.) and cork oak (*Q. suber* L.). Both species of oak cover large, continuous areas, and extensive gypsy moth outbreaks have occurred throughout Spain.

A survey of the natural enemies of the gypsy moth in Spain had been conducted earlier by the Laboratorio de Fauna Forestal Española from 1913–36, and the following parasites were recorded: *Anastatus disparis, Apanteles "vitripennis," A. liparidis, A. porthetriae, A. melanoscelus, Brachymeria intermedia, Meteorus "versicolor," Trichogramma* sp., *Exorista larvarum, E. segregata, Compsilura*

concinnata, Blepharipa pratensis and *Parasetigena silvestris* Robineau-Desvoidy.

Between 1960 and 1965, the same species were recovered from studies conducted in Toledo-Avila and Guadalajara Provinces as those included on the 1913-36 list, with the exception of *Trichogramma* sp., *P. silvestris, C. concinnata* and *B. pratensis.* The additional parasite species *O. kuvanae* and scavenger Diptera (*Sarcophaga uliginosa* Kramer, S. *tuberosa* Kramer and *Agria affinis* (Fallén)) were present in these recently monitored study areas.

Most of the detailed observations on individual species of parasites were made on a 3,000-ha evergreen oak forest. The gypsy moth caused heavy defoliation, especially in the last 3 years of the study, even though the parasites were generally active, because presumably unfavorable conditions (storms, hail, and cold) were more injurious to the parasites than to the host. Only *A. "vitripennis"* was an important parasite of early-stage larvae and *E. segregata* and *B. intermedia* of later stage larvae and pupae.

Apanteles "vitripennis" was considered the most important of the six species of *Apanteles,* although not as important as *E. segregata* or *B. intermedia.* In some cases, percentages of parasitism of over 78 percent were found, while the average was only 15 percent. A total of 12,000 cocoons and 16,000 cocoons was sent to Moorestown in 1964 and 1965, respectively.

Data collected during the 5 years of the study indicate that *E. segregata* is one of the most important (13 to 35 percent parasitism) parasites of the gypsy moth in the areas studied in Spain. An additional 5 percent parasitism should be added relating to host larvae parasitized by *Exorista* that did not reach pupation and died; these, therefore, were not considered in pupal counts. A total of 43,000 *E. segregata* puparia were forwarded to the ARS quarantine facility, then at Moorestown, N.J.

In the southern areas of the study forest, *Brachymeria intermedia* adults were obtained from host pupae from mid-June onward and in the colder areas (the northwest) 2 weeks later. *Brachymeria* was one of the most effective parasites (14 percent to 65 percent parasitism) of the gypsy moth and especially abundant

over the years, and it was not hyperparasitized. A total of 31,900 adults was sent to the ARS facility in 1963 and 1964. However, high mortality (18 percent to 20 percent of those shipped) reduced the numbers eventually released in Connecticut.

Other parasites of the gypsy moth studied during the project included *A. disparis,* a native egg parasite with one generation per year and up to 9.2 percent parasitism; *O. kuvanae,* an imported egg parasite with several generations per year and up to 20 percent parasitism; and *E. larvarum,* with a range of parasitism from 1.5 percent to 3 percent.

Hyperparasitism was observed on Diptera and *Apanteles.* In 1960, the percentage of parasitized dipterous puparia was very high, especially in Avila-Toledo, where it reached a maximum of 62 percent. *Monodontomerus aereus* (Walker) was practically the only species causing the high mortality of puparia. From 1961 to 1965, only approximately 2 percent of the tachinid puparia were attacked by *M. aereus* and *Dibrachys* sp.

Forest Service Sponsored Public Law 480 Projects in India: 1961–71
Richard C. Reardon

Project No. A7–FS–8, "Survey for Natural Enemies of the Gypsy Moth," was conducted from July 25, 1961 to July 24, 1966. V. P. Rao of the Indian Station, Commonwealth Institute of Biological Control, Bangalore-6, India, was principal investigator of this project. The following is a brief resume of the final report of the project. Shipments of natural enemies resulting from this project and its replacement project are shown in table 6.1-1 in the Recent History section.

The gypsy moth found in India (Kashmir, Kotgarh and Kulu) (fig. 6.1-1) is *Lymantria obfuscata* (Walker) and not *L. dispar* (L.), although the biologies of both species are similar. Natural enemies— over 100 species of parasites, 8 predators and 1 nematode—were reared from *L. obfuscata* and other lymantriids.

During the survey, 5 egg parasites, 33 larval parasites, and 11 pupal parasites of *L. obfuscata* were recorded. Unfortunately, species determinations were

not available for many of the parasites recovered, and only those with a specific designation and considered "important" are listed below.

Egg parasites: *Anastatus ?"kashmirensis"* was abundant at Kulu and Srinagar, with four to five generations per year, and sometimes up to 25 percent parasitism. *A. disparis,* with three to four generations per year, averaged 2 to 5 percent parasitism at Srinagar (fig. 6.1–1).

Larval parasites: *Apanteles liparidis* was one of the most common species attacking larvae (parasitism ranged between 0.5 to 7 percent) in Kashmir and Kotgarh. *Rogas indiscretus* was very prominent in Kulu and Kotgarh, with parasitism of 22 to 24 percent reported at Kulu. *Compsilura concinnata, Exorista rossica,* and *Palexorista* spp. parasitized 15 to 25 percent of late-stage larvae.

Pupal parasites: *Brachymeria "euploeae"* (5 to 15 percent parasitism) and *B. intermedia* (12 to 15 percent parasitism) were the most important pupal parasites.

Project No. A7–FS–51, "Evaluation of Hymenopterous Parasites of the Gypsy moth and Study of the Behavior of Promising Species," replaced the previous project and was conducted from March 1, 1967, to August 31, 1972. V. P. Rao (to February 24, 1971) and P. R. Dharmadhikari (from February 25, 1971), Indian Station, Commonwealth Institute of Biological Control, were principal investigators. The following is a brief summary of the final report of this project.

The parasite evaluation program had several objectives: In 1967, the selection of species that might be useful against *L. dispar* in the United States, and from 1968 to 1971, the development of life tables for *L. obfuscata* on *Salix* and *Populus* spp. in four different localities (two dry and two marshy) in Kashmir.

Major population reduction in each generation of the gypsy moth was in the egg stage; 80 percent of the eggs were lost on both *Populus* and *Salix* in the dry and marshy areas. The parasite *A. disparis* accounted for between 0.2 and 6 percent of this mortality and unknown factors and eclosion failure made up the remainder. During the first larval stage, the mortality

was 1 to 12 percent on both these hosts and in different habitats; no deaths were attributed to parasites. In the second larval stage, the mortality on *Populus* was 1 to 26 percent, while on *Salix* it was 1 to 25 percent. Mortality due to parasites (*Apanteles* spp.) ranged between 0.3 and 16 percent, regardless of the host plant and habitat. In the third larval stage, the maximum population decline was up to 28 percent for *Populus* in dry areas. Between 0.6 and 19 percent of the mortality was attributable to *Apanteles* spp. Mortality in the fourth larval stage was less than that in the fifth and sixth stages considered together and ranged between 11 percent and 62 percent on both host plants and in both habitats.

The parasites *Apanteles* spp., *Palexorista* spp., and *E. rossica* accounted for between 0.7 and 11 percent of the mortality. In the fifth and sixth larval stages, mortality fluctuated between 33 and 96 percent, and *E. rossica* and *Palexorista disparis* Sabrosky accounted for 26 percent of the mortality. The decline of the pupal population was 17 to 95 percent, and percent parasitism by *B. intermedia* and five species of ichneumonids ranged between 3 and 73 percent. Among the pupal parasites, *B. intermedia* gave appreciable parasitism of *L. obfuscata* while other pupal parasites (*Coccygomimus* spp.) were useful as supplementary parasites. The decline in the population of *L. obfuscata* at the end of each generation ranged between 89 and 99 percent, regardless of the host plant or habitat. Nevertheless, the residual population of *L. obfuscata,* with a high rate of reproduction, gave rise to high populations in the next generation.

The only parasite that showed an appreciable degree of control of *L. obfuscata* during the 4 consecutive years of the study was *E. rossica.* On the whole, the performance of *Apanteles* spp. was not promising.

During this study, 150 *A. disparis,* 2,240 *Apanteles* spp., 75 *B. intermedia,* 10 *Coccygomimus* spp., 765 *E. rossica,* 2,540 *Palexorista* spp., 1,532 *Monodontomerus aereus* and 1,058 *R. indiscretus* were sent to the ARS Beneficial Insect Research Laboratory quarantine facility, then at Moorestown, N.J.

Figure 6.1-1—*Map of India showing important localities surveyed for gypsy moth and allied Lymantriids and their natural enemies.*

Forest Service Sponsored Public Law 480 Projects in Yugoslavia: 1967-77

Richard C. Reardon

Project No. E30–FS–9, "A Biological Method of Control of Injurious Insects *Lymantria dispar* L. and *Diprion pini* L.," was conducted from February 1, 1967, to December 31, 1971. Dr. Konstantin Vasić, Institute of Forestry and Wood Industry of Serbia, Kneza Viseslava 3, Belgrade, Yugoslavia, was principal investigator of this project. The following is a brief resume of the final report of the project. No shipments of natural enemies were made as a direct result of this project or its replacement projects.

This investigation provided significant laboratory data for: A method of obtaining large quantities of host larvae throughout the year for the purpose of mass- production of *Apanteles;* the optimal age limit of the larvae suitable for rearing *Apanteles;* and optimal methods for rearing parasites and the most favorable methods of parasitization. In addition, preliminary field studies indicated that the efficiency of individual species of *Apanteles* varies with geographical region and climate. For example, *A. liparidis* has been rare in the plains and southern regions of Yugoslavia, whereas *A. "solitarius"* is most often found in poplar and other forests of the plains. *A. porthetriae* and *A. melanoscelus* are the most abundant and efficient of the *Apanteles* spp.

Thirty species of hyperparasites have been recovered; the most significant were *Dibrachys cavus* (Walker), *Eurytoma* spp. (especially *verticillata* (F.) and *"strigifrons"* Thomson), and *Hemiteles pulchellus* Gravenhorst.

Project No. E30–FS–79, "The Effectiveness of Certain Parasites and Predators to Control Gypsy Moth," was conducted from April 1, 1972, to March 31, 1975. K. Vasić of the Institute of Forestry and Wood Industry of Serbia, was again principal investigator.

The hymenopterous parasites of the gypsy moth found in Yugoslavia are numerous (about 50 species). Two species parasitize eggs, six species attack second through third-stage larvae, four species attack fourth through sixth-stage larvae, and the others are para-

sites of pupae or are hyperparasites. Among the parasites of second- and third-stage larvae, most are from the genus *Apanteles* (*porthetriae*, *"solitarius," melanoscelus*, *ocneriae*, and *liparidis*), although *"solitarius"* is considered a synonym of *melanoscelus* (Muesebeck 1978).

Studies of *Apanteles* spp. recovered from the gypsy moth during the period of gradation were carried out in three localities in Yugoslavia. *Apanteles* spp. were very numerous in forests of low density and with a lot of light. During the 3-year investigation, the most numerous species of *Apanteles* were *porthetriae* (44.7 percent), *"solitarius"* (30.2 percent), and *melanoscelus* (25.1 percent). *A. "solitarius"* was the most numerous at the edge of the forest (38.2 percent) and the least in the middle (10.5 percent). *A. melanoscelus* was the most effective in the middle of the forest (36.9 percent), while *A. porthetriae* was more numerous in orchard situations (52.3 percent).

Investigations proved that *Apanteles* spp. were the most active at the top of the crown and that their efficacy decreased toward the base of the crown. At the top, the rate of parasitism was in the range of 1.0 to 2.7 percent; in the middle, from 0.6 to 2.1 percent; and at the base of the crown, from 0.6 to 1.3 percent.

Hyperparasitic species attacking *Apanteles* spp. were numerous; a total of 12 different species of secondary parasites and two tertiary parasitic species was recovered. The most significant reduction role of hyperparasites was during 1972, when half of the recovered cocoons was decimated by the activity of these hyperparasites. During the next 2 years, secondary parasites destroyed about 26 percent and 31 percent, respectively, of primary parasites.

Investigations of the alternate hosts for all *Apanteles* spp. did not provide satisfactory results. Although large numbers of caterpillars of different species from the families Geometridae, Noctuidae, Lasiocampidae, Lymantriidae, Tortricidae and Pyralidae were collected and reared, no recoveries were made of any *Apanteles* species known to parasitize the gypsy moth.

Species of parasites from the genus *Meteorus* (for example, *versicolor* and *pulchricornis*) are rare para-

sites of the gypsy moth in Yugoslavia. Both species were recovered but always as a single specimen and in few locations.

Project No. E30–FS–79–JB–47 (a 2-year extension of Project No. E30–FS–79), "Investigation of the Possibility to Use a Pure Line of Gypsy Moth with Shortened Diapause and *Apanteles* spp. for Biological Control of Gypsy Moth," was conducted from December 22, 1975, to November 30, 1977, with K. Vasić again serving as principal investigator. The following results are summarized from a 1-year progress report (December 1975–December 1976), as a final report was not available for review.

Studies to determine rates of parasitism by *Apanteles* spp. in different biotypes were continued in one of the localities in Serbia. As previously determined, *Apanteles* spp. were the most effective in places with a lot of light. During 1976, the *Apanteles* rate of parasitism of gypsy moth was between 3 and 12 percent. The rate of parasitism by *Apanteles* spp. was greatest at the top of the trees (11.5 percent) and decreased toward the base of the crown (3.4 percent).

Tachinid species were often recovered from the gypsy moth, and rates of parasitism were generally uniform (25 to 28 percent) in all biotypes. Tachinids were the most numerous at the base of the crown (36 percent) and least in the middle (17 percent).

An increase in parasitism was obtained by release of *A. "solitarius"* that were mass reared in the laboratory. One thousand cocoons were placed in a 100-m² plot of oak woodland in 1975 when gypsy moth larvae were in the first instar. In 1976, parasitism by *A. "solitarius"* increased 4.9 percent in the release site as compared with 0.3 percent decrease in the control site. The rate was also higher (12 percent) in comparison with parasitism for other species of *Apanteles* in the release area.

Agricultural Research Service Sponsored Public Law 480 Project in Yugoslavia: 1972–75
John J. Drea, Jr.

Project No. E30–ENT–35, "Inventory of Natural Enemies of Gypsy Moth in Yugoslavia and the Col-lection and Shipment of Live Materials to the United States," was conducted from May 15, 1972, to November 14, 1975. Dr. Konstantin Vasić, Institute of Forestry and Wood Industry of Serbia, Kneza Viseslava 3, Belgrade, served as principal investigator of this project. The following is a summary of the reports of the project. Shipments of natural enemies resulting from the project are shown in table 6.1–1 in the Recent History section.

The gypsy moth is a well-known pest of forests in Yugoslavia. It is endemic in the country, with periodic outbreaks occurring in various localities. In other regions, the moth is known to exist at more or less low-density populations. Because of the intensive research with *Lymantria dispar* (L.), a great deal of information is available concerning the dynamics of the insect and its associated natural enemies (Sisojević 1955, Vasić 1957). The background of information available and the history of continued low populations of the moth in some areas indicated that a study of *L. dispar* under these conditions might uncover a factor or factors responsible for maintaining the pest at these low levels.

The project was to be 3 years in duration covering three full seasons of moth activity. The object was to collect as many larvae and pupae as possible from *low* host-density populations to obtain one or more species that may be responsible for maintaining the host at a low density.

Through the use of burlap bands and bark flaps attached to trees, the presence of low-density populations of *L. dispar* was determined at 15 localities, 14 of which are indicated in figure 6.1–2. Of these, 10 sites were studied during each of the 3 years of the project. Change in population levels prevented continual studies at all sites.

For the 14 localities sampled in 1973, the total larval/pupal parasitism of 5,288 hosts was 7.8 percent; in 1974 it increased to 8.9 percent of 7,421 hosts from 13 localities; in 1975, parasitism was 11.5 percent of 8,853 hosts from 11 localities. Approximately 36 different species of parasites and predators were found associated with *L. dispar* during this study (table 6.1–2). Because of distances involved and the variation in
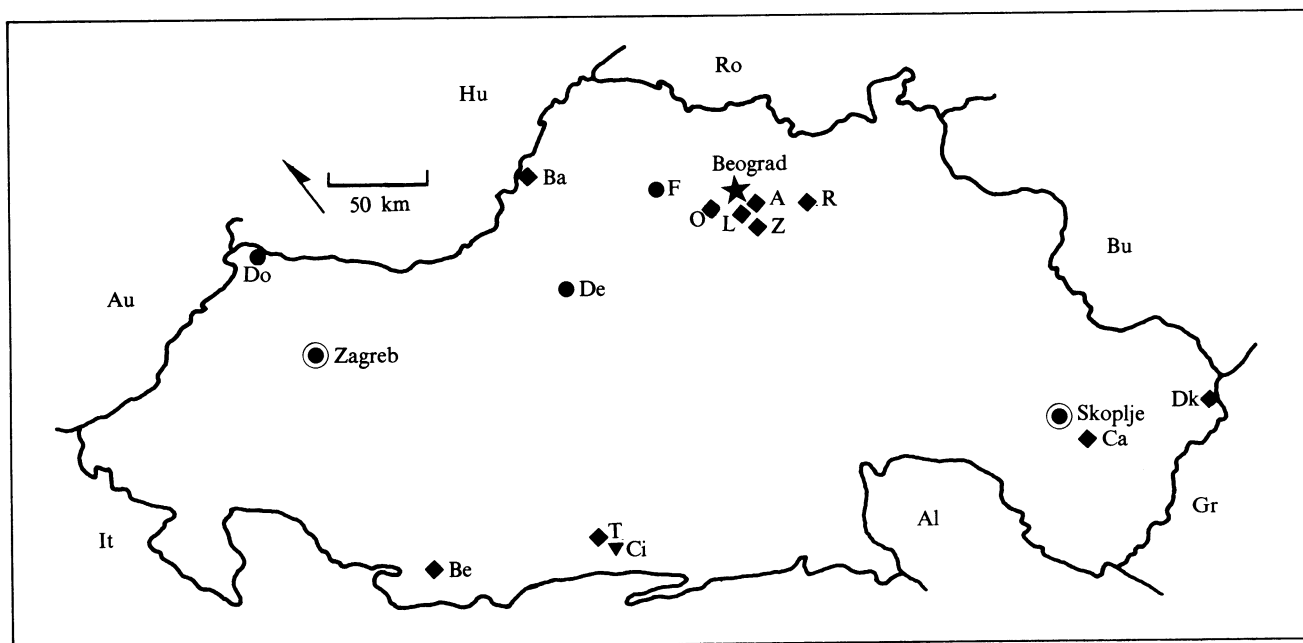
Figure 6.1-2.—*Collecting sites for low density population of* Lymantria dispar *in Yugoslavia, 1972-75.*
A—Avala; Ba—Backi Breg; Be—Benkovac; Ca—
Carevic Prilep; Ci—Citluk; De—Dervnta; Dk—Demir
Kapijva; F—Fruska Gora; L—Lipovica; O—Obrez;
R—Rogot; T—Tihal Jina; Z—Zovljak. ◆—*Studied*
for 3 years; ●—*Studied for 2 years;* ▼—*Studied for*
1 year.

host populations each year, only 10 localities were sampled every year.

Identifications were made by K. Vasić for the Hymenoptera, P. Sisojević for the Tachinidae, and by M. Slamcekova, Nitra, Czechoslovakia, for the Sarcophagidae.

No specific studies were made with the egg parasites *A. disparis* and *O. kuvanae*, although they were recovered from egg masses. Both species are well established in the United States (Hoy 1976), and the latter species is a recent introduction into Yugoslavia (Tadic and Bincev 1959). A dermestid, *Megatoma pici*[*=ruficornis*], was very common on egg masses and destroyed a large number of eggs, but because of the uncertain status of dermestids regarding introduction, the species was not sent to the United States. Considering the effect of dermestids on egg masses as was noted during this study and in a similar one in

Morocco, this group of insects may merit further consideration. This would apply especially to those species whose biologies and specificity are still unknown.

Braconids of the genus *Apanteles* were the most important Hymenoptera attacking the larval stages of the host. The overall parasitism by this group, however, never attained 6.0 percent except at Backi Breg, where parasitism was 7.2 percent in 1974 and 21.4 percent in 1975. *Meteorus* sp., probably *pulchricornis* (Wesmael), was negligible in all localities.

Despite the fact that nine species of ichneumonids were recorded, only *Phobocampe* spp. and *Coccygomimus* spp. appeared to be anything more than incidental parasites. Species of *Phobocampe* were recovered from all localities except Lipovica, although not in every year of the study. Overall parasitism never attained 5.0 percent at any site. *Coccygomimus* spp. were recovered from Benkovac, Carević Prilep and

Table 6.1-2.—*Parasites and predators associated with* Lymantria dispar *in Yugoslavia, 1972-75*

Braconidae	Apanteles lacteicolor Viereck	A. ocneriae Ivanov
	A. liparidis (Bouché)	A. praepotens (Haliday)
	A. melanoscelus (Ratzeburg) (=A. solitarius (Ratzeburg))	A. porthetriae Muesebeck
		Meteorus sp.
		B. intermedia (Nees)
Chalcididae	Brachymeria sp.	
Encyrtidae	Ooencyrtus kuvanae (Howard)	
Eupelmidae	Anastatus disparis Ruschka	
Ichneumonidae	Casinaria tenuiventris (Gravenhorst)	Phobocampe disparis Viereck
	Coccygomimus instigator (F.)	P. "pulchella Thompson"
	C. turionellae (L.)	Lymantrichneumon disparis (Poda)
	Ephialtes compunctor (L.)	Theronia atalantae (Poda)
	E. rufatus (Gmelin)	
Tachinidae	Blepharipa pratensis (Meigen)	Exorista larvarum (L.)
	Blondelia nigripes (Fallen)	Palexorista inconspicua (Meigen)
	Carcelia separata (Rondani)	Parasetigena silvestris (Robineau-Desvoidy)
	Compsilura concinnata (Meigen)	Zenillia libatrix (Panzer)
Sarcophagidae	Kramerea schuetzei (Kramer)	Agria affinis (Fallen)
Carabidae	Calosoma sycophanta (L.)	C. inquisitor (L.)
Dermestidae	Megatoma pici Kalik [=a synonym of M. ruficornis (Aube)]	
Silphidae	Xylodrepa quadripunctata (L.)	

Tihal Jina but in very low numbers, with overall parasitism never reaching 1.0 percent.

The chalcids, *Brachymeria* spp. were essentially nonexistent and exerted no apparent control of the hosts in those areas where pupae were obtained.

There were eight species of tachinids recovered during the study. All species are common and important parasites of *L. dispar*, with the possible exception of *Zenillia libatrix*, which may appear on *L. dispar* but is primarily a parasite of the browntail moth, *Nygmia phaeorrhoea* (Donovan) (Sabrosky and Reardon 1976). The tachinids were by far the most effective

group of parasites, but except for rates of 24.3 percent at Obrez and 15.4 percent at Avala, the overall parasitism for the 3-year study was only about 6.4 percent at the 10 localities.

Two or 3 species of sarcophagids were associated with the gypsy moth, but no specific information was obtained as to the status of these species as parasites. Furthermore, Leonard (1974) records that sarcophagids are less efficient than tachinids, and the actual attack on living hosts by the group is still uncertain.

The carabids *Calosoma sycophanta* and *C. inquisitor* were common at most localities, but no specific studies were made with these species. The silphid *Xylodrepa quadripunctata* was an occasional predator of the moth larvae.

In none of the localities where *L. dispar* existed at low densities was there a species or species-complex that appeared to be specific to low host populations. Furthermore, the overall parasitism in most of the sites sampled was below that obtained in similar studies in other parts in Europe. The species recovered were known also from medium to high host-density populations, or appeared to be incidental parasites with little, if any, impact on the host population. As the host density increased from 1973 to 1975, the percentage parasitism increased at some localities and decreased at others. Any conclusions of this type would require a more intensive study over a longer period of time. However, it is apparent that a search among low populations for a species or complex that may control *L. dispar* is not promising.

Agricultural Research Service Sponsored Public Law 480 Project in Morocco: 1972-75

Franck Hérard and John J. Drea, Jr.

Project No. F12-ENT-1, "Collection of Predators and Parasites of Gypsy Moth, Lygus Bug, and Certain Other Economically Important Insect Pests," was conducted from July 19, 1972, to July 20, 1975. Dr. Alain Fraval, Institut Agronomique et Veterinaire Hassan II, B.P. 704, Rabat, Morocco, served as principal investigator for the project, and Franck Hérard served as main entomologist. The following is a summary of the reports of the project. Shipments of natu-

ral enemies resulting from the project are shown in table 6.1-1 in the Recent History section.

The gypsy moth was first recognized as such in Morocco in 1921, although the "processionary moth of oak" of an earlier reference was actually *Lymantria dispar* (L.). However, the insect was known to the natives for many years. Although outbreaks cause damage at times, they are sporadic, and the insect is not considered a serious pest. Nevertheless, according to Boudy (1958), defoliation of cork trees, *Quercus suber* L., by the moth for 3 consecutive years causes a 50-percent deficiency in growth, resulting in a 16-percent loss at the time the cork is harvested.

The fauna associated with *L. dispar* in Morocco was first discussed by Ferrière (1927) and later by de Lépiney (1930). Their studies indicated there was a complex of species attacking the gypsy moth which under ideal conditions could produce up to 80 percent parasitism. De Lépiney (1930) recorded an average of 15 to 20 percent parasitism for the period 1926–29 in the Mamora cork oak forest near Rabat. However, other than these two studies, there was very little additional research done up to the present with the parasites of *L. dispar* in Morocco.

This project was established in 1972 for a 3-year period. The study was to include surveys to determine the distribution of the moth in Morocco and collections of host material to obtain natural enemies for shipment to the United States. Although parasites and predators from north Africa had never been introduced into North America, the few references available indicated that the parasitism in this new region could be high at times. Therefore, a project was initiated to obtain species or races for introduction that might be effective in North America, especially in view of the gradual southward spread of the moth.

Because of the warm climate of Morocco, the eggs of *L. dispar* hatch in February and March, with eclosion lasting for up to 2 months. Larval development averages about 50 days, followed by a 2- week pupal period. Adult moths are found from June through August, depending upon the altitude.

Most of the studies made were in stands of the cork oak, the principal host of the moth. In addition to occasional attack on other species of *Quercus, L. dis-*

par may damage *Pyrus malus* L., *P. communis* L., *P. mamorensis* Trabut, *Prunus domestica* L., and *Cydonia vulgaris* Person. In periods of outbreak it may attack *Eucalyptus* spp., *Aleuretis fordii* Hemsl., *Cistus salvifolius* L., *Schinus molla* L., and even *Pinus halepensis* Miller.

The distribution of the moth, including new and previously unrecorded locality records, was determined using the trapping technique described by Fuester et al. (1975) (fig. 6.1-3). The moth occurred throughout Morocco wherever a suitable host was found, with the heaviest infestation being in the Mamora and Sahel Forests of the western and northern parts of the country.

A list of the natural enemies associated with *L. dispar* is presented in table 6.1-3. Not all specimens were identified to species because some failed to emerge from the pupal stage or because their status has not yet been determined.

Egg parasites were obtained by field examination and by collecting and isolating egg masses in individual containers. The most abundant egg parasite was the encyrtid *O. kuvanae*. It was recorded in all stands of *Q. suber* but never from eggs collected on *Q. ilex* L. According to de Lépiney (1930), *L. dispar* had no egg parasites prior to the introduction of *O. kuvanae* in 1927 except for a few individuals of *Telenomus phalaenarum* (Nees) (Scelionidae) and one specimen of *Ooencyrtus masii* (Mercet) found in 1926. During this study, four species of *Telenomus* were associated with gypsy moth egg masses but no *T. phalaenarum* were recovered. Also, several specimens of the scelionid *Gryon* sp. were recovered from egg masses but the host relationship was not clear.

Predators were quite abundant on the egg masses. The effect of the species of dermestids, *Trogoderma versicolor, Anthrenus verbasci,* and *Dermestes* sp., was considerable in all localities studied. Hérard (1978) reported that up to 50 percent of the egg masses laid in cork oak in the Mamora forest in Morocco were destroyed by one or more of these dermestids. *Tenebroides maroccanus* of the family Trogositidae was also an abundant egg predator on the masses, as was *Akis bacarozzo* (Tenebrionidae).

Larval and pupal parasites were obtained by col-
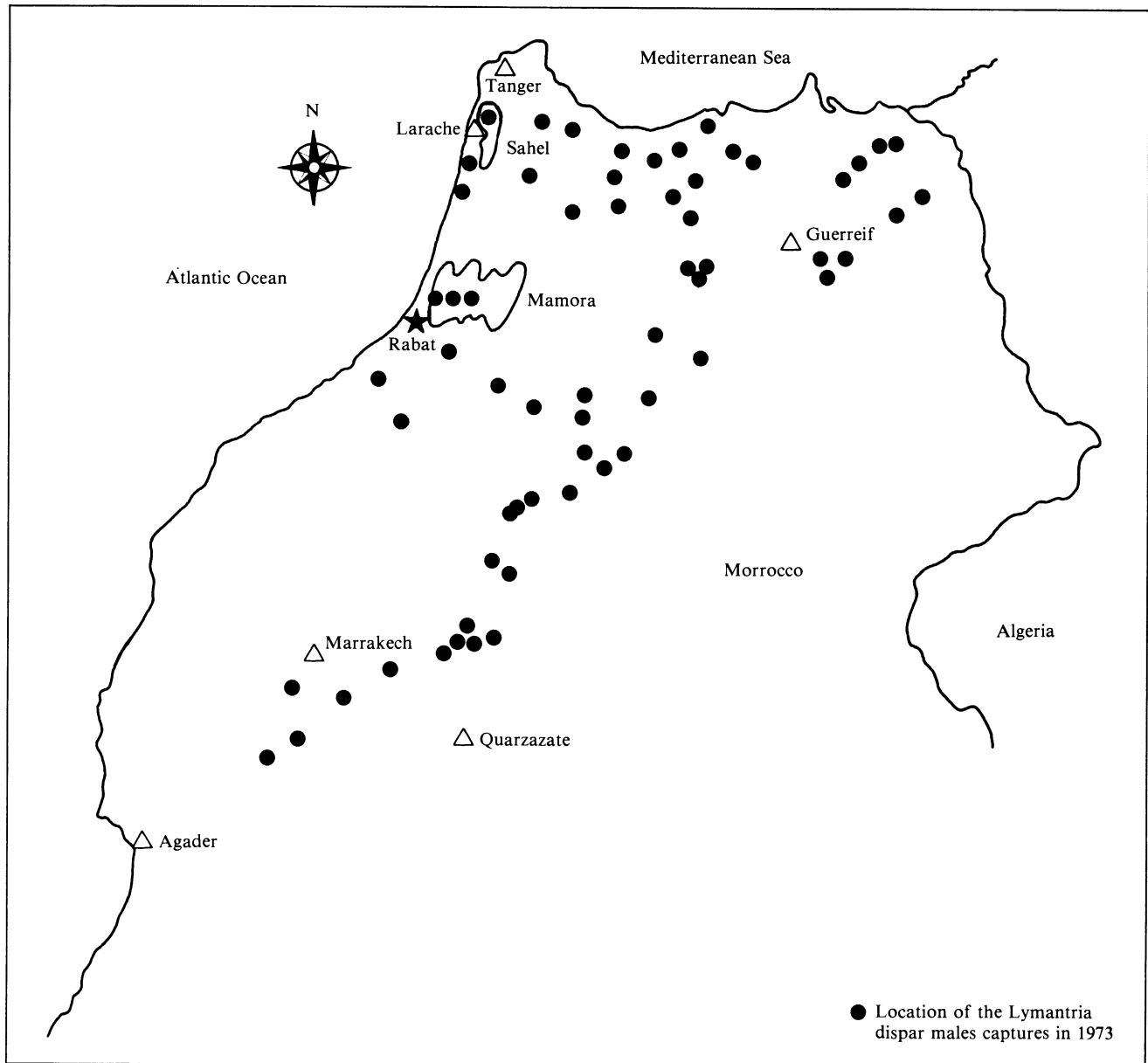
Figure 6.1-3—*The distribution of* Lymantria dispar
*(L.) in Morocco as determined by the use of traps
containing a synthetic sex pheromone.*

Table 6.1-3—*Species of parasites and predators associated with Lymantria dispar in Morocco, 1973-75*

Braconidae	*Apanteles lacteicolor* Viereck	*A. porthetriae* Muesebeck
	A. melanoscelus (Ratzeburg) (=*solitarius* (Ratzeburg))	*Meteorus pulchricornis* (Wesmael)
Ichneumonidae	*Coccygomimus* sp.	*Vulgichneumon* sp. (=*Melanichneumon* sp.)
	C. instigator (F.)	
	C. turionellae moraguesi (Schmiedeknecht)	Ichneumonid spp. indet.
Chalcididae	*Brachymeria intermedia* (Nees)	
Encyrtidae	*Ooencyrtus kuvanae* (Howard)	
Formicidae	*Crematogaster scutellaris* (Olivier)	
Scelionidae	*Gryon* sp.	*Telenomus* spp.
Tachinidae	*Carcelia separata* (Rondani)	*Palexorista inconspicua* (Meigen)
	Compsilura concinnata (Meigen)	Tachinid spp. indet.
Carabidae	*Calosoma sycophanta* (L.)	*Scarites occidentalis* Bedel
Dermestidae	*Anthrenus verbasci* (L.)	*Trogoderma versicolor* (Creutzer)
	Dermestes sp.	
Trogositidae	*Tenebroides maroccanus* Reitter	
Tenebrionidae	*Akis bacarozzo* (Schrank)	
Araneida		

lecting the host in the field and by holding the insects in the laboratory until either the host insect completed its normal development or a parasite emerged from the host. The host larvae were fed on small oak branches and leaves.

The family Braconidae was represented by three species of *Apanteles*. *A. melanoscelus* was abundant from hosts on both *Q. suber* or *Q. ilex*. *A. porthetriae* was recovered only from hosts on *Q. suber*. *A. lactei-color* was also found on *Q. suber* but in low numbers. This is a new record for Morocco. The only other braconid found was *Meteorus pulchricornis*, recovered primarily from hosts on *Q. suber* but occasionally from *Q. ilex*.

The only representative of the family Chalcididae was *Brachymeria intermedia*. In general it was rare on

absent from the collections except for the sample from Larache where 11.4 percent of the host pupae produced this species.

The family Ichneumonidae had several species associated with *L. dispar*. There were two or possibly three species of *Coccygomimus*, a species of *Vulgichneumon*, and at least two species of undetermined ichneumonids. However, their total effect on the host larval or pupal populations was slight. One species, *C. turionella moraguesi*, was eventually mass produced and released in the United States.

In general the tachinids were also of little importance except for two small samples collected at Jbel Tazzeka and Chaouen, where parasitism was high. *Palexorista inconspicua* was the most abundant species, with *Compsilura concinnata* of secondary importance. *Carcelia* was present but in negligible numbers. Overall, the tachinids all together produced a total parasitism of less than 1.0 percent.

The carabid *Calosoma sycophanta* was present in Morocco but not in the numbers found in other areas (such as Corsica) where it has been credited with the control of some outbreaks of *L. dispar*.

Hyperparasites were very abundant and appeared to exert a considerable effect on the primary species. Cocoons of *Apanteles* spp. were heavily attacked by *Dibrachys* sp., *D. cavus* (Walker), three species of pteromalids, "*Agilis* sp.," "*Drinoderus* sp.," a species of chalcidid, and even *O. kuvanae* on occasion. *Meteorus pulchricornis* was the host of *Gelis areator* (Panzer) and an unidentified pteromalid.

The fauna associated with *L. dispar* in Morocco was not as rich as originally suspected. In fact, there did not appear to be any specific natural enemy that was responsible for the control of the moth in the country. Nevertheless, sufficient numbers of several species were obtained and shipped to the United States. In 1975, the overall parasitism produced by all the parasites recovered from the host larvae was only 1.22 percent. This is considerably lower than any region in Europe where similar studies were made as reported elsewhere in this chapter by Fuester et al. As a result, the government of Morocco has considered establishing an introduction program to import,

primarily from Europe, those species missing from the complex associated with *L. dispar.*

It would be well to consider importing the ichneumonid *Casinaria tenuiventris* Gravenhorst and especially the tachinids *Parasetigena silvestris* (Robineau-Desvoidy) and *Blepharipa pratensis* (Meigen), which are among the most important parasites of the gypsy moth in Europe. Although *O. kuvanae* is widespread in Morocco, the egg parasite *Anastatus disparis* Ruschka might also be considered as a possible candidate.

Miscellaneous Overseas Activities in Spain and Yugoslavia: 1967–70

Richard C. Reardon and Jack R. Coulson

As noted in the Recent History section, the Plant Protection Division (PPD) (now the Plant Protection and Quarantine Programs of Animal and Plant Health Inspection Service) and the Division of Plant Industry of the New Jersey Department of Agriculture (NJDA) have both been involved in the overseas collection of gypsy moth natural enemies.

In spring 1967, two PPD inspectors traveled to Spain to collect gypsy moth pupae to replenish the supply of natural sex lure. As a result, 594,000 female pupal tips were returned to the United States for processing (Warner 1978). In the process, approximately 10,000 *Exorista segregata* (Rondani) puparia were recovered from field-collected host pupae and supplied to the ARS Beneficial Insects Research Laboratory, then at Moorestown, N.J. A total of 1,350 flies emerged at that laboratory and were brought or sent to the PPD (now APHIS) Methods Development Center at Otis Air Base, Mass. Approximately 259 died en route, and the living flies were released at Sandwich, Mass., with the exception of about 10 females, which were used to initiate a small laboratory culture at Otis.

In summer 1968, approximately 500,000 female pupal tips were shipped to the United States as well as 870 cocoons of *Apanteles,* later determined to be *A. porthetriae,* with the possible inclusion of some *A. liparidis.* The 316 adults emerging from this material were sent to Otis to initiate a laboratory culture.

In 1969, collections yielded a total of 815,200 female abdominal tips. In addition, 8,000 *E. segregata* puparia and 1,912 *A. porthetriae* cocoons were brought to the ARS Moorestown laboratory, and the emerging adults from this material were sent to the Pennsylvania, New Jersey, and New York Departments of Agriculture and to Otis for release. A total of 817 *A. porthetriae* and 5,755 *E. segregata* adults were shipped by the Moorestown facility in 1969.

In 1970, NJDA provided funds for the study and collection of gypsy moth natural enemies in Yugoslavia by a Rutgers University graduate student, Kevin J. Hackett. The following is a brief summary of Mr. Hackett's Master of Science Thesis.

The study was undertaken to determine if the parasites that had been imported previously into the United States from areas of high gypsy moth populations were the same as those associated with low host populations. The Danube River Valley near Belgrade, Yugoslavia, was chosen as the study location. This area is similar climatically and ecologically to areas of gypsy moth infestations in New England, New Jersey, and Pennsylvania. Six sites were chosen for dominant tree species and range of gypsy moth density. Burlap bands were used for larval collection, although other parasite material was collected from the foliage in order to give an unbiased estimate of percent parasitism.

The *Apanteles* species appeared to respond in two different ways to the density of gypsy moth populations. First, in stabilized areas a greater diversity of parasites appeared responsible for the higher incidence of parasitism. Second, although diversity may have increased in high-density areas, multibrooded parasites were able to take advantage of the abundance of gypsy moth larvae and appeared to be dominant over single-brooded ones.

Various *Phobocampe* spp., but possibly only *P. disparis* (Viereck), were the only ichneumonids recovered. Their presence appeared related to factors other than host density. The overwintering cocoons were heavily hyperparasitized.

The tachinid "*Carcelia gnava*" (probably *C. separata*) appeared of only minor importance in the con-

trol of the gypsy moth. *Compsilura* sp. (probably *concinnata*) was abundant in areas of low to medium host density, although it probably responded to both the density of the gypsy moth and to the relative abundance of other necessary alternate and overwintering hosts. *Exorista larvarum* (L.) was the most abundant parasite in the area of highest gypsy moth population and relatively scarce in all other areas.

From the six areas surveyed, *A. liparidis* (Bouché), *A. melanoscelus,* and *Compsilura* appeared to be low-density parasites, while *E. larvarum* was abundant at high densities of the gypsy moth. Populations of *Apanteles ocneriae* Ivanov and *Phobocampe* spp. appeared not related to gypsy moth density. Only two egg parasites (*O. kuvanae* and *A. disparis*) were observed.

The shipments of natural enemies sent to the NJDA via the ARS Beneficial Insect Research Laboratory quarantine facility from Yugoslavia in 1970 are shown in table 6.1-1 in the Recent History section.

Miscellaneous Overseas Activities in Japan, the U.S.S.R., and India: 1972-77
Jack R. Coulson

In 1972, funds were made available to ARS for overseas explorations for gypsy moth natural enemies. In addition to the initiation that year of work at the ARS European Parasite Laboratory and the sponsorship of two Public Law 480 projects (discussed previously), ARS entered into a contract with the European Station of the Commonwealth Institute of Biological Control (CIBC) for some initial studies of the gypsy moth and its natural enemies in Japan. The objectives of the contract with CIBC were to collect parasites of low-density gypsy moth populations in Japan for shipment to the United States and to provide information relative to the possibilities of future gypsy moth biological control studies in Japan. Travel to Japan was undertaken by Dr. H. Zwölfer of the CIBC European Station during June, July, and August of 1972. The information gathered by Zwölfer was presented in a report (Zwölfer 1972), which included some recommendations for potential future studies in Japan. The following is a brief summary of that report.

The report discussed the biology and ecology of Japanese gypsy moth populations, including reference to the various "races" known to occur there from earlier work by Goldschmidt (1929, 1932, 1933). Also discussed was the occurrence of past outbreaks in different parts of Japan. These outbreaks generally last from 1 to 3 years, with periods between outbreaks averaging 10 years. The last serious outbreaks occurred in 1952-53 and 1961-63, both on the northern island of Hokkaido (Yogo 1963). Zwölfer's report noted that outbreaks on the central island of Honshu are much less severe than on Hokkaido, and that the gypsy moth exists in low densities on the southern island of Kyushu, where outbreaks do not occur. He listed 26 parasites and 4 predators of the gypsy moth taken from the natural enemy catalog of Yasumatsu and Watanabe (1964, 65) and a few recent unpublished sources. Brief biological notes on 16 of the parasite species were given. In addition to these, the report noted the importance of bird predation of gypsy moth larvae (citing unpublished data of K. Furuta, National Forest Experiment Station, Sapporo) and of fungus and nucleopolyhedrosis virus diseases as regulating factors in Japan. It was noted that *Apanteles liparidis* is one of the most important parasites in Japan, because of its ability to develop two generations during the gypsy moth larval period and to locate and attack low-density populations. This species and several other Japanese parasites utilize *Dendrolimus* spp. (Lasiocampidae) as alternate and overwintering hosts in Japan; the utilization of these alternate hosts tends to increase the parasite reservoir.

Zwölfer suggested that further field studies aimed at quantitative analysis of mortality factors be conducted, preferably in Kyushu where gypsy moth populations are stabilized at low endemic levels. He also suggested further investigations of the life histories and ecology of such scarce and localized parasites as *Meteorus japonicus* Ashmead (?= *pulchricornis* (Wesmael)), *Rogas lymantriae* Watanabe, and *Lymantrichneumon disparis* (Poda), and further study of the genetics of the gypsy moth in Japan for possible genetic control purposes in the United States. He expressed doubt that a natural enemy survey of the conventional type would produce any hitherto unde-

tected control agents of the gypsy moth in Japan but noted that such a survey would be useful in providing further needed information on distribution, ecology, and relative importance of Japanese natural enemies of the gypsy moth.

Zwölfer was unable to conduct field collections in Japan but did arrange for the shipment in 1972 of one lot of *A. liparidis* to the United States by Dr. K. Katagiri of the Government Forest Experiment Station, Tokyo (see table 6.1-1).

In 1972, the U.S. Department of Agriculture entered into a formal agreement with the U.S.S.R. Ministry of Agriculture. One provision of this agreement concerned an exchange of natural enemies of specific insect and weed pests as requested by the respective countries. Included among the material requested from the U.S.S.R. by ARS were natural enemies of the gypsy moth. Specific species requested included several egg parasites (*Ooencyrtus* and *Telenomus* species) of the gypsy moth in the U.S.S.R. and *Hexameris "albicans"* (Siebold), a nematode parasite complex reported to have caused up to 60 percent mortality of gypsy moth larvae in one area of the U.S.S.R. in 1951 (Artyukhovskii 1953). Although a number of natural enemies have been received from the U.S.S.R. as a result of the exchange agreement, only one shipment relates to the gypsy moth. In 1976, a shipment of juvenile specimens of the nematode were received, most of which unfortunately were dead upon arrival (table 6.1-1, Recent History). The agreement also provided for exchange of scientific personnel for travel in the respective countries. Two attempts to send U.S. entomologists to the U.S.S.R. for survey and collection of gypsy moth natural enemies have been unsuccessful. Attempts are continuing to obtain gypsy moth natural enemies from the U.S.S.R. under this agreement.

In 1975 and again in 1976 and 1977, ARS entered into contracts with the Indian Station of the CIBC for collections in India of several specific parasites found during the course of earlier Public Law 480 projects sponsored by the Forest Service that were requested by researchers of the Connecticut Agricultural Experiment Station (AES) and the Forest Service Northeastern Forest Experiment Station in Connecticut.

Collections all 3 years were made by G. Ramaseshiah of the CIBC station in the Kotgarh and Kulu Valley areas of Himachal Pradesh state and the Srinagar area of Jammu and Kashmir state of northern India. Shipments of natural enemies resulting from these collections are shown in table 6.1-1 in the Recent History section.

In 1975, requests were made for the collection of the larval parasite *Rogas indiscretus* and the egg parasite identified in earlier Public Law 480 reports as *Anastatus ?kashmirensis* Mathur but generally discussed in the earlier reports as "*A. bifasciatus* (Fonsc.)" (Rao 1966, Dharmadhikari 1972). The strain of *Rogas* being cultured in the United States as a result of earlier shipments from India was believed to have lost its capacity for diapause, and fresh stock was desirable in the hope of establishing a diapausing strain of the species. The *Anastatus* was desired for culture and study relative to the potential of the species for field release in the United States. Collections in 1975 made from *L. obfuscata* on various species of *Quercus, Alnus, Populus,* and *Salix* in the three collections areas were unfortunately initiated rather late in the season (June 10 to August 7). Consequently, there was heavy hyperparasitism of the *Rogas* cocoons collected, and no specimens of *Rogas* emerged from the 181 cocoons sent to the ARS quarantine facility from India in 1975. However, a sufficient number of *Anastatus* emerged from the 3,150 egg masses shipped to initiate laboratory cultures in Connecticut and at the ARS quarantine facility in Delaware. This species was identified as *Anastatus* sp. near *kashmirensis* Mathur by G. Gordh, ARS–SEL. Additional taxonomic studies are required before the proper name of this species can be definitively determined; the species is thus labeled *A. ?kashmirensis* throughout this chapter.

In 1976, the contract called for additional collections of *Rogas indiscretus* and *Anastatus ?kashmirensis*. However, specimens of the various *Apanteles* species and of the *Brachymeria "euploeae* Westwood" reported in the earlier Indian Public Law 480 reports were also requested, as well as of any specimens of hosts killed by pathogens found during the collections. The *Apanteles* and *Brachymeria* were requested by the Connecticut AES; the pathogens were

requested by the Forest Service laboratory in Connecticut. Collections similar to those of 1975 were conducted in the three areas of India from April 9 to August 7, 1976. As a result, additional *Anastatus* and 75 adult *Rogas indiscretus* were obtained in the ARS quarantine facility from 5,670 egg masses and 565 *Rogas* cocoons, respectively, sent from India, for shipment to the Forest Service laboratory in Connecticut. However, the great majority of the 1,592 field-collected *Apanteles* cocoons collected and shipped to the ARS quarantine facility from India were hyper-parasitized, and no adult *Apanteles* were available for the Connecticut AES. Also, all adult *Brachymeria* sent from India were received dead. Very few *Brachymeria* adults emerged in quarantine from any of the host pupae suspected to be parasitized by *Brachymeria;* those few that emerged were identified as *B. intermedia* (Nees). Thus, no Indian material was sent to the Connecticut AES in 1976.

In 1977, the same species, with the exception of *Anastatus,* were requested from India. Collections were made of *Apanteles* species cocoons in the Kashmir Valley from *L. obfuscata* on *Salix* and *Juglans* species from April 9 to May 29, and of *Apanteles, Rogas,* and *Brachymeria* in the Kulu Valley from June 1 to July 1, 1977. Again, the great majority of the 3,887 field-collected *Apanteles* cocoons sent to the ARS quarantine facility from these collections were found to be hyperparasitized; 100 percent of the later shipments were hyperparasitized. However, from some of the early shipments of cocoons received from Kashmir, enough *Apanteles* adults emerged for shipment to Connecticut, where they were successfully cultured. These were identified by P.M. Marsh of SEL as *A. ?liparidis, A. ?ruidis* Wilkinson, and *A. lacteicolor* Viereck. A culture of *A. ?ruidis* was also later established at the Pennsylvania Bureau of Forestry laboratory at Harrisburg. From the 257 *Brachymeria*-parasitized host pupae received from the Kulu Valley, 61 adult *Brachymeria* emerged; these were tentatively identified as "*euploeae*" and were sent to the Connecticut AES where a successful culture was established. Only two *Rogas indiscretus* adults were obtained from the 494 heavily hyperparasitized, Kulu-collected *Rogas* cocoons received, and these were sent to Con-

necticut. Some entomogenous fungus and NPV material was also received in the ARS quarantine facility from India in 1977 and shipped to Forest Service and ARS pathologists.

As with *Anastatus ?kashmirensis,* some taxonomic questions exist concerning the proper names for the *Apanteles* and *Brachymeria* species received from India in 1977, and the species now in culture in the United States are labeled *A. ?ruidus* and *B. "euploeae"* throughout this chapter. Additional comments on the work that has been conducted in the United States on the four Indian species imported in 1975-77 (*Rogas indiscretus, Anastatus ?kashmirensis, Brachymeria "euploeae,"* and *Apanteles ?ruidis*) can be found in other sections of this chapter.

Agriculture Research Service Overseas Explorations, 1972-77

Explorations in Europe and Iran by the ARS European Parasite Laboratory: 1972–77

Roger W. Fuester, John J. Drea, Jr., Francis Gruber, and Franck Hérard

Introduction

Although much of the previous exploration for natural enemies of the gypsy moth, *L. dispar,* was conducted in Europe (Burgess and Crossman 1929, Howard and Fiske 1911), there were several reasons to believe that further explorations there might be useful:

• The rapidity of modern air transport and recent improvements in insect rearing techniques have increased the probability that some of the parasites of European origins that failed to become established might become established if release attempts were repeated at the present time.

• The location, observation, and collection of those species of natural enemies attacking low-density host populations (earlier work consisted of locating outbreaks of the gypsy moth), in the hope that such enemies might result in improved control of gypsy moth in North America.

- The augmentation of existing gene pools of the New World populations of those species already established in North America, to increase the effectiveness of species such as *Phobocampe disparis* (Viereck).

- In addition, the possibility of finding new or previously untried species of natural enemies.

Objectives

In view of the aforementioned considerations, the staff of the European Parasite Laboratory (EPL) initiated a program in 1972 with six primary objectives:

- A fundamental starting point in any classical biological control project is to find out what natural enemies attack the pest in its place of origin. The first objective, therefore, was a basic inventory of parasites. In this case, it was known that France was the place of origin of the North American population of gypsy moth (Burgess and Baker 1938).

- The second objective was to collect and ship natural enemies of gypsy moth to the United States for study and possible release as part of the expanded gypsy moth program. Such shipments were to include those species already established in North America, those that had been introduced in previous attempts but which had failed to become established, and any new species of natural enemies that might be found. Whereas living natural enemies are indispensable for implementation of a biological control program, this objective was considered to be the most important of all. Because providing living material was the primary goal, much time was spent collecting and rearing hosts to obtain parasites for shipment. These activities precluded making detailed observations on host density, impact of vertebrate predators, or other factors influencing populations of gypsy moth.

- Observations and collections were to be made in different regions of Europe in order to minimize the possibilities of poor climatic adaptation and overlooking a potential control agent that was rare or absent in any given region. Moreover, observations on regional differences in abundance of a given species were expected to provide information as to what type of climate would suit it best.

- Because previous overseas collections were nearly always made at the sites of heavy infestation of gypsy moth (Crossman and Webber 1924), an effort was to be made to collect in areas of low-, medium-, and high-density populations of gypsy moth.

- Because different species of parasites tend to be abundant at different times of the season, observations and collections were to be made throughout the season. In addition to minimizing the possibility of missing a potentially effective control agent, this approach would make it possible to ascertain the optimum time for release and recovery of a given species of parasite.

- To obtain information on the relative importance of the various species of parasites, observations were to be made on percentage parasitism. When such data are analyzed in conjunction with climate, forest type, and host density, information essential to the release program in North America may be obtained.

Regions Explored

Most of the observations were made in six different geographic regions (fig. 6.1–4): southeastern France, central France, eastern Austria, northern Bavaria, central Poland, and northern Iran. These regions were chosen because they differed considerably in climate and flora (table 6.1–4), and thus provided sufficient geographical and ecological diversity to constitute a well-balanced exploration program. No observations were made in southeastern Europe or on the Iberian Peninsula because this would have unnecessarily duplicated studies in progress or already completed by scientists working on Public Law 480 projects in Spain and Yugoslavia. Because it was desirable to obtain natural enemies from regions having diverse climates, observations were made for only one or two seasons in a given region.

Egg Parasites

Prospections for egg parasites of *L. dispar* were made in southeastern France (1972–73), eastern Austria (1974–75), western and central Poland (1975),
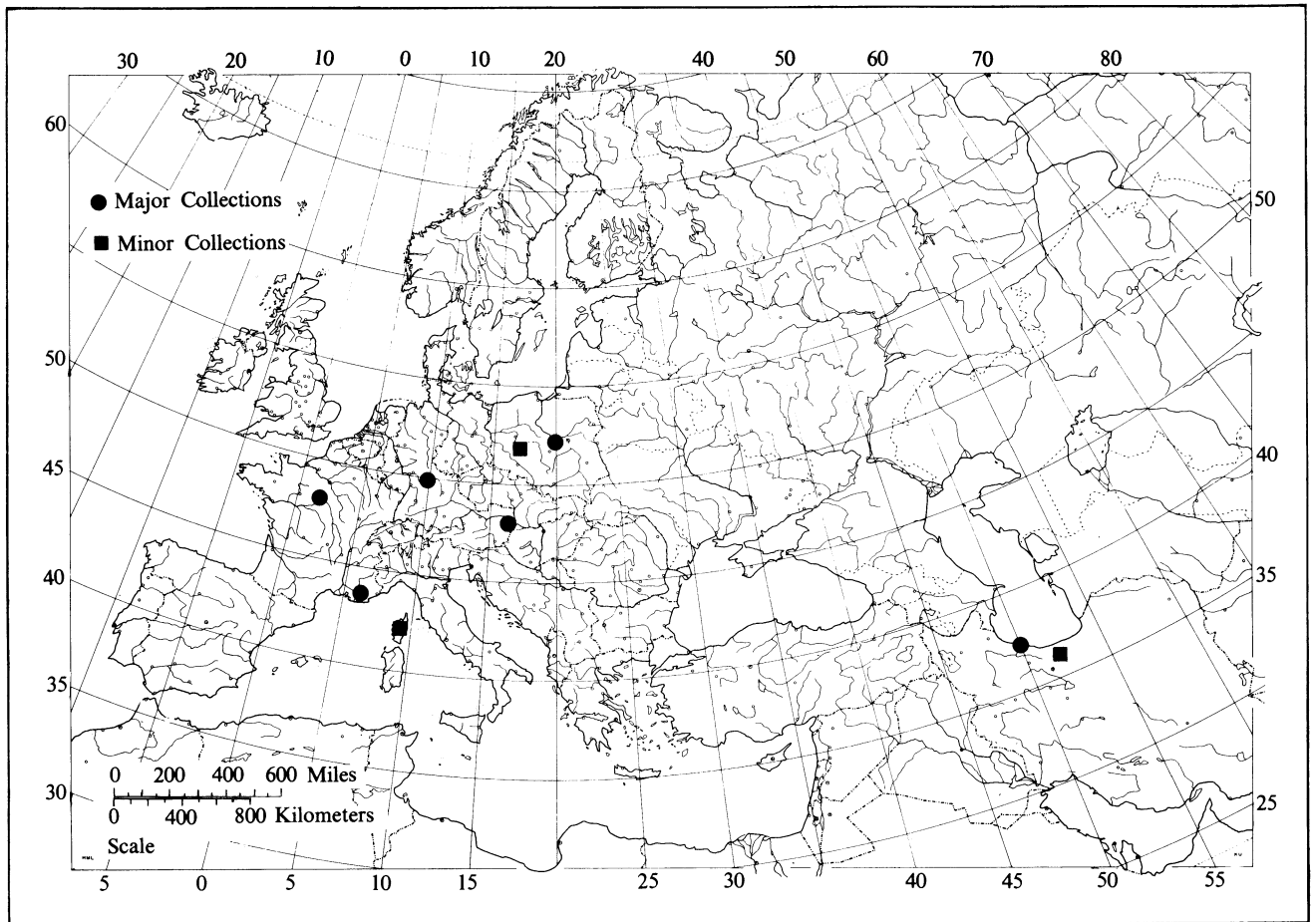
Figure 6.1-4.—*Sites of study and collection of natural enemies of gypsy moth in Europe and Iran.*

and northern Iran (1976–77). For recovery of egg parasites, host egg masses were field collected from late summer to midwinter. After collection, the masses were placed individually in plastic dishes 6 cm wide and 2 cm deep with tight-fitting lids and held at 20°–25° C in the laboratory for parasite emergence. After 30 days, emergence of parasites not in diapause was assumed to be terminated, and the samples were placed in a refrigerator at 5°–8° C until they could be dehaired and counted using an apparatus similar to that described by Tardif and Secrest (1970). Some egg masses contained parasites in diapause that emerged after they had been chilled.

A total of 278 egg masses was collected at 35 different localities in southeastern France during the period September 19–October 2, 1972. Larger collections were possible in 1973, and a total of 1,237 was obtained from 63 localities from August to October. The eupelmid *Anastatus disparis* Ruschka was clearly the dominant egg parasite on a regional level being recovered at 19 localities during 1972 and at 33 localities during 1973. The encyrtid *Ooencyrtus kuvanae* (Howard) was generally subdominant, being recovered at only six localities in 1972 and at 11 localities in 1973, but was the dominant species recovered at seven of these localities. Several

Table 6.1-4.— *Types of climates and preferred host plants of* Lymantria dispar *in different regions explored*

Region	Type of climate	Preferred host plants
Southeastern France	Mediterranean	*Quercus ilex* L.
		Quercus pedunculata Ehrh.
		Quercus suber L.
Central France	Maritime	*Quercus* spp.
		Robinia pseudoacacia L.
Eastern Austria	Continental	*Quercus* spp.
		Carpinus betulus L.
		Robinia pseudoacacia L.
		Corylus colurna L.
Northern Bavaria	Continental	*Quercus pedunculata* Ehrh.
		Carpinus betulus L.
		Tilia
Central Poland	Continental	*Tilia*
		Pyrus malus L.
Northern Iran (Caspian region)	Subtropical	*Alnus*
		Populus
		Prunus
		Gleditsia
		Quercus
		Carpinus

scelionids, *Telenomus* spp., *Gryon* spp., and *Idris* sp., were recovered in very low numbers during 1972, but not at all during 1973. Because of personnel limitations, the percentage parasitism in the egg masses collected during 1972 could not be determined. During 1973, total parasitism (by *A. disparis* and *O. kuvanae* combined) in the different samples ranged from 0.1 to 72.0 percent. Mean total parasitism for all samples combined (498,840 eggs) was 15.6 percent. Because most samples contained eggs from which parasites had already emerged prior to collection, it was impossible to determine accurately the percentage of eggs parasitized by each species.

Collections of egg masses were made in the Burgenland province of eastern Austria during March and August of 1974 and during February and September of 1975. The only parasite recovered

consistently was *A. disparis*. Only one broken specimen of *O. kuvanae* was recovered from an egg mass of *L. dispar* collected in oak-hornbeam forest near Girm on February 22, 1975. In addition, very low numbers of *Telenomus* spp. were obtained from several localities in Burgenland during February 1975. Rearings from material collected during August 1974 showed that parasitism by *A. disparis* in individual masses ranged from 0.0 to 57 percent and averaged 19 percent.

Because of the extreme scarcity of *L. dispar* in Polish forests during 1975, all egg-mass collections in Poland were made on roadside plantings of linden (*Tilia*), where host density was often rather high (10 or more egg masses per tree). Although several hundred egg masses of *L. dispar* were collected at four different localities (Racot, Twiew, Alexsandrowo, and Nieborów) during early September 1975, no egg parasites ever emerged from any of these samples. Because the egg masses were handled in the same manner as those collected in Austria only a few days later, one can only assume that parasites were very rare at or absent from the collecting sites.

Prospections for egg parasites in Iran were made only in the Caspian Sea region. Egg-mass collections were made during July and December 1976 and February and March 1977 near Amol in the eastern region, and during December 1976 and February and March 1977 near Bandar Pahlevi in the western region. The midwinter collections were made by Dr. Abai of the Plant Pest and Diseases Research Institute in Tehran, who forwarded the material to the EPL by air freight. The only egg parasite recovered consistently was *A. disparis,* but parasitism by this species was very low in both regions. In addition, three *Telenomus* spp. and a *Gryon* sp. were recovered in very low numbers. Several other insects were associated with egg masses of gypsy moth: The cryptophagid *Telmatophilus caricis* (Olivier), a phalacrid (*Stilbus* sp.), a throscid (*Trixagus* sp.), and a lyonetiid (*Bucculatrix* sp.). However, no evidence was obtained that any of these insects actually fed on eggs of *L. dispar.*

In most of the regions studied, *A. disparis* was clearly the most important egg parasite of *L. dispar.*

Although *O. kuvanae* was locally abundant and an important mortality agent at several localities in southeastern France, it was either very rare or not found in the other regions surveyed. This species was first introduced into Spain in 1923 (Clausen 1956); from there, it spread into France. It is probable that *O. kuvanae* has not yet dispersed throughout its potential range in Europe and may become more important in the future. In most areas surveyed, mean total parasitism of eggs was 15–20 percent. In general, this is substantially lower than the 25–50 percent reported by Dowden (1961) and Doane (1968) for New England, where *O. kuvanae* is generally the dominant parasite.

Specimens belonging to three different genera of Scelionidae were recovered from egg masses of gypsy moth, but none was ever obtained in large numbers or observed attacking gypsy moth eggs in the laboratory. As eggs of arthropods other than *L. dispar* are sometimes found in egg masses of gypsy moth, it is possible that some or all of these scelionids are not bona fide parasites of the target pest. This is likely to be the case concerning the genera *Gryon* and *Idris*, the members of which are generally parasites of Hemiptera and spiders, respectively (Marsh 1976).

Larval Parasites

Because the maximum number of parasites for shipment was desired, normal collecting and rearing techniques were used to estimate the percentage of hosts containing developing parasites. Usually, a sample consisted of 200 larvae, but at some low host-density localities, 50 or fewer larvae were found.

In 1972–73, studies of larval parasites of gypsy moth were conducted in southern France. In 1972, considerable time was spent surveying south central and southeastern France for populations of *L. dispar*. As a result, many locations were visted only once, and none was sampled regularly throughout the season. Nevertheless, a total of 48 samples comprising 11,857 larvae was taken from 27 different localities. In April 1973, prior to egg hatch, a temporary laboratory was established at Le Luc in the Var Department, permitting a more methodical operation. Five study

sites were chosen in the vicinity, and larval collections were made at approximately weekly intervals at each site. In addition, numerous spot samples were taken on an irregular basis at other localities in the region. A total of 179 samples (36,142 larvae) was taken during 1973. The results obtained in 1972 were incomplete and did not differ materially from those obtained in 1973, so only the latter will be discussed in detail. Weekly parasitism data for the collections made in 1973 appear in table 6.1–5.

In 1973, hatch started about May 1, and collections were started on May 3. During the early season, May 3–20, when most of the larvae were in the first and second instars, the braconids *Apanteles porthetriae* Muesebeck and *Apanteles melanoscelus* (Ratzeburg) were clearly the most important parasites (table 6.1–5), and mean total parasitism was about 19 percent. In midseason, May 21–June 10, when third- and fourth-instar larvae were prevalent, no parasite was clearly dominant, and mean total parasitism was rather low, about 9 percent (table 6.1–5). In late season, June 11–July 11, when fifth- and sixth-instar larvae were prevalent, the tachinid *Blepharipa pratensis* (Meigen) was the most effective parasite, and mean total parasitism was relatively high, about 32 percent (table 6.1–5). Several other tachinids were also locally abundant at this time: *Carcelia separata* (Rondani), *Palexorista inconspicua* (Meigen), and *Parasetigena silvestris* (Robineau-Desvoidy). In addition, the braconid *Apanteles liparidis* (Bouché) parasitized 10 percent or more of the late-instar larvae at a few localities. Although several other parasites were recovered from larvae of gypsy moths (table 6.1–5), none appeared to be nearly so important as those cited above. It is perhaps noteworthy that the rarest parasite was the polyphagous tachinid *Compsilura concinnata* (Meigen), which is a very common and important parasite of *L. dispar* in North America (Burgess and Crossman 1929, Clausen 1956, Dowden 1962, Hoy 1976, Sabrosky and Reardon 1976, Tigner 1974*a*).

Observations on larval parasites of the gypsy moth were made at the Forêt d'Orleans in central France during 1974–75. However, sampling throughout the season was possible only in 1976. During 1974, four

Table 6.1–5.—*Parasitism of larvae of* Lymantria dispar (L.) *in southeastern France, 1973*

Parasite species	Dates collected and percent parasitized									
	May 3–May 6	May 7–May 13	May 14–May 20	May 21–May 27	May 28–June 3	June 3–June 10	June 11–June 17	June 18–June 24	June 25–July 1	July 2–July 11
Apanteles porthetriae	12.2	12.5	6.5	3.3	1.4	0.9	0.6	0.2	0	0
A. melanoscelus	6.1	6.4	6.7	1.2	1.9	2.7	3.4	1.5	.4	0
A. liparidis	0	.3	.2	.4	.2	.4	.6	2.7	2.7	1.5
Meteorus spp.	.2	1.3	1.2	1.4	.8	.4	.4	.5	1.1	1.3
Casinaria spp.	.4	.4	.3	.1	<.1	<.1	0	0	0	0
Phobocampe disparis	0	.9	.5	.4	.6	.7	.1	0	0	0
Hyposoter tricoloripes	0	<.1	0	<.1	<.1	<.1	<.1	0	0	0
Carcelia separata	0	0	<.1	.1	.5	.9	1.8	1.7	2.2	10.1
Palexorista spp.	0	0	0	<.1	.2	.2	.2	.6	1.4	14.0
Parasetigena silvestris	0	0	.2	.2	.7	.7	3.2	2.8	3.5	1.3
Blepharipa pratensis	.2	.6	.2	1.3	2.5	4.1	14.5	23.4	27.1	9.0
Compsilura concinnata	0	0	0	0	0	<.1	<.1	<.1	0	.1
Total parasitism	19.1	22.5	15.8	8.4	8.9	11.2	24.8	33.4	38.3	37.3
Host larvae collected	509	3,212	5,831	4,170	5,157	3,884	4,259	4,787	3,183	1,150

collections were made (May 30 and July 8, 11, and 15), but the population of *L. dispar* was very low, and only 97 larvae were collected altogether. In 1975, populations were higher, and a total of 1,111 larvae were collected from May 30 to July 17. In 1976, collections were made at two stations, Bellegarde and St.-Denis-de-l'Hôtel, where 1,292 and 2,494 larvae were collected, respectively, from May 10 to July 12. Table 6.1–6 summarizes the parasitism of the larvae collected. During all 3 years, the ichneumonid *Hyposoter tricoloripes* (Viereck) was the most important parasite in the region. Whereas *H. tricoloripes* usually emerge from late third-instar larvae of *L. dispar*, parasitism by this species peaked in late May or early June: 16 percent on May 30, 1974; 31 percent on June 5, 1975; and 25 percent (Bellegarde) and 11 percent (St.-Denis-de-l'Hôtel) on May 20, 1976. Thereafter, parasitism dropped rather sharply. Nevertheless, it is suspected that *H. tricoloripes* usually destroys 10–20 percent of the third-instar larvae in this region. None of the remaining parasites recovered consistently parasitized high percentages of larvae. However, *Phobocampe disparis* (Viereck) was the dominant parasite at one of the stations (St.-Denis-de-l'Hôtel) in 1976 (table 6.1–6) and parasitized 14 percent of the larvae collected there during May

Table 6.1–6—*Parasitism of larvae of* Lymantria dispar (L.) *at Forêt d'Orleans, France, 1974–76*

Parasite species	Year collected and percent parasitized			
	1974	1975	1976[1]	1976[2]
Apanteles porthetriae	0	0	0.2	<.1
A. melanoscelus	0	.3	2.5	.2
A. liparidis	2	.1	.9	.2
Meteorus sp.	1	0	0	0
Casinaria tenuiventris (Grav.)	0	0	.2	0
Phobocampe spp.[3]	2	1.7	1.1	6.0
Hyposoter tricoloripes	7	6.1	6.8	2.5
Carcelia separata	0	0	0	<.1
Parasetigena silvestris	4	1.1	2.3	.9
Blepharipa pratensis	1	3.0	1.0	.7
Compsilura concinnata	0	.1	0	<.1
Blondelia nigripes (Fall.)	0	0	0	<.1
Total	17	12.3	14.9	10.3

[1]Sizable collections made at Bellegarde.
[2]Sizable collections made at St.-Denis-de-l'Hôtel.
[3]Dominant species *P. disparis*.

10–20. The only tachinids recovered all 3 years were *P. silvestris* and *B. pratensis*. As in southeastern France, parasitism by *C. concinnata* was negligible (table 6.1–6). Mean total parasitism was below 20 percent all

3 years (table 6.1-6), primarily because parasitism of late instars by Tachinidae was rather low.

During 1974-1975, larval collections and rearings were made in the Burgenland Province of eastern Austria. Weekly samples were taken at nine sites in 1974 and at 10 sites in 1975 (seven sites were sampled both years). A total of 93 samples (12,326 larvae) was taken in 1974, and 116 samples (13,684 larvae) were taken in 1975. Weekly parasitism data for the collections made in 1974 and 1975 appear in tables 6.1-7 and 6.1-8, respectively. During 1974, *B. pratensis* and *P. silvestris* were clearly the dominant larval parasites with the former species predominating at most localities. Whereas larvae parasitized by *B. pratensis* were fairly abundant early in the season (table 6.1-7), the phenology of *B. pratensis* in eastern Austria differed from that observed in southeastern France (table 6.1-5). The most important hymenopterous parasites were the following: *A. liparidis, A. melanoscelus, P. disparis,* and *Meteorus* spp. Total parasitism at the different localities ranged from 18 to 41 percent and averaged 30 percent.

During 1975, the population of *L. dispar* dropped considerably at most localities. A comparison of tables 6.1-7 and 6.1-8 shows that *P. silvestris* was clearly the dominant larval parasite in this region during 1975, but that parasitism by *B. pratensis* was much lower during 1975 than in 1974. Whereas mean parasitism by *B. pratensis* was 10.6 percent in 1974, it was only 1.2 percent in 1975, a ninefold drop in effectiveness. These results are similar to those obtained in Yugoslavia by Sisojević (1959), who observed an eightfold decrease in abundance of *B. pratensis* in 1958 in relation to the previous year, which was coincident with an increase in abundance of *P. silvestris.*

In 1975, the most effective hymenopterous parasites were *A. liparidis, P. disparis,* and *H. tricoloripes* (table 6.1-8). Parasitism by *A. melanoscelus* dropped significantly in 1975 (tables 6.1-7 and 6.1-8), but not so sharply as in the case of *B. pratensis.* The parasitic nematode *Hexameris "albicans"* (Siebold) was recovered during both years (tables 6.1-7 and 6.1-8) but was more important during 1975 when

mean parasitism was 1 percent. Maximum parasitism was 11 percent at one locality. Detailed observations on this nematode as a parasite of *L. dispar* were presented by Artyuhovskii (1953) and Drea et al. (1977).

During 1975, total parasitism at the different localities ranged from 8 to 45 percent, averaged 24 percent, about 6 percent lower than during 1974. The most notable drops in parasitism were observed at those sites where the host population had declined markedly. It appears that the effectiveness of most species of parasites was greatly reduced at extremely low densities. This was especially true in the case of *B. pratensis.* Only *P. silvestris* and *A. liparidis* were recovered consistently at localities having low populations during both years of the study in eastern Austria.

In 1974, samples of gypsy moth larvae were obtained from northern Bavaria through the efforts of Drs. Schwenke and Skatulla of the University of Munich, who made collections every 2 weeks at an outbreak in an oak plantation near Würzburg, Germany. Six samples totaling 4,847 larvae were obtained through their cooperation; rearing results are shown in table 6.1-9. Parasitism by *A. melanoscelus* was low early in the season but increased to high levels late in the season. In five seasons of work on gypsy moth, this is the only locality observed where high parasitism was effected by the second brood of *A. melanoscelus.* Another important parasite at Würzburg was *P. disparis,* which parasitized about 12 percent of the larvae during midseason and an average of 7.6 percent over the season. The phenology of *P. silvestris* followed the same general pattern as elsewhere in Europe: Parasitism by this species was very low early in the season but rose to over 20 percent in late June and early July. Although *B. pratensis* parasitized about 10 percent of those larvae collected early in the season, it was less effective thereafter, and mean parasitism by the species was 5.7 percent. Several other parasites, including the nematode *H. "albicans"* were reared from larvae collected at Würzburg (table 6.1-9), but none of these appeared to have a significant impact on

Table 6.1-7.—*Parasitism of larvae of* Lymantria dispar (L.) *in Burgenland, Austria, 1974*

Parasite species	Date collected and percent parasitized												
	4/23 to 4/29	4/30 to 5/6	5/7 to 5/13	5/14 to 5/20	5/21 to 5/27	5/28 to 6/3	6/4 to 6/10	6/11 to 6/17	6/18 to 6/24	6/25 to 7/1	7/2 to 7/8	7/9 to 7/15	7/16 to 7/22
Apanteles porthetriae	0	0	0.4	0.2	0.3	0.2	0.6	0.4	0.5	0.5	0	0	0
A. melanoscelus	0	.3	.4	2.4	.1	1.5	2.2	2.2	2.5	2.3	1.5	.2	0
A. liparidis	.7	.4	2.4	1.4	1.6	.9	.2	.4	.3	3.4	3	7.6	1.6
Meteorus spp.	0	.3	0	1.0	1.6	3.1	3.7	1.7	2.5	1.8	3.9	2.0	2.2
Casinaria tenuiventris	0	0	.4	.2	.2	.4	.2	.2	0	0	0	0	0
Phobocampe spp[1]	.7	3.8	.7	2.0	3.0	3.4	2.8	2.2	2.3	1.0	.4	0	0
Hyposoter tricoloripes	0	.1	1.6	.4	.1	<.1	.9	.5	.1	.3	0	0	0
Carcelia separata	0	0	0	<1.1	0	<.1	.4	.4	.2	<.1	.1	0	.3
Parasetigena silvestris	.7	.8	.5	2.1	10.0	15.0	18.1	14.4	11.0	17.5	10.1	17.9	7.7
Blepharipa pratensis	19.5	11.8	14.8	12.0	11.4	11.8	10.3	10.2	11.2	5.0	9.9	3.7	5.8
Tachinidae spp[2]	0	0	0	<.1	0	0	.1	.3	.3	.4	.3	.5	.6
Hexamermis "albicans"	0	0	.4	.5	.2	.4	.3	1.0	.3	.6	.3	0	0
Total parasitism	21.4	17.5	21.4	22.3	28.7	37.0	39.7	33.8	31.1	32.7	29.3	31.9	18.3
Host larvae collected	154	732	552	1,204	920	1,060	1,039	1,268	1,045	2,205	812	1,008	312

[1]Includes *Phobocampe disparis* (Viereck), which was dominant, and an apparently undescribed species.

[2]Includes *Peribaear tibialis* (R.-D.), *Blondelia nigripes* (Fallén), *Compsilura concinnata* (Meigen), and *Siphona samarensis* (Villeneuve).

Table 6.1-8.—*Parasitism of larvae of* Lymantria dispar (L.) *in Burgenland, Austria, 1975*

Parasite species	Date collected and percent parasitized											
	4/30 to 5/6	5/7 to 5/13	5/14 to 5/20	5/21 to 5/27	5/28 to 6/3	6/4 to 6/10	6/11 to 6/17	6/18 to 6/24	6/25 to 7/1	7/2 to 7/8	7/9 to 7/15	7/16 to 7/22
Apanteles porthetriae	0	0.3	<.1	<.1	0	0	0	0	0	0	0	0
A. melanoscelus	1.1	.3	2.0	.5	.3	.4	.8	1.2	.4	0	0	0
A. liparidis	0	1.3	3.6	2.8	3.2	1.6	1.6	6.4	5.6	9.4	3.3	0
Meteorus pulchricornis	0	0	.2	.8	.9	.6	.1	.3	.1	0	0	0
Casinaria sp.	0	0	0	<.1	0	0	0	0	0	0	0	0
Phobocampe disparis	.4	4.2	5.6	2.7	4.1	4.1	4.8	1.6	.4	0	0	0
Hyposoter tricoloripes	0	0	.6	1.7	4.3	2.9	1.6	.4	0	0	0	0
Carcelia separata	0	0	0	<.1	0	<.1	0	0	0	0	0	0
Parasetigena silvestris	0	.2	1.0	10.8	13.2	15.9	18.8	15.1	17.8	13.4	19.2	11
Blepharipa pratensis	4.7	.5	.4	.1	.7	.5	.7	.5	1.1	2.7	1.3	2
Hexamermis "albicans"	0	3.5	2.2	1.9	2.4	1.5	1.4	1.4	.5	1.1	0	0
Total parasitized	6.2	10.5	15.7	21.3	29.1	27.6	29.8	27.0	25.9	26.7	23.8	13
Total hosts	274	960	1,580	2,200	1,588	1,600	1,908	1,779	1,078	698	151	48

Table 6.1–9—*Parasitism of larvae of* Lymantria dispar (L.) *at an oak plantation near Würzburg, Germany, 1974*

	Date collected and percent parasitized					
Parasite species	April 23	May 8	May 20	June 5	June 20	July 3
Apanteles melanoscelus	1	1	1	8	22	32
Meteorus sp.	0	0	0	0	<1	0
Casinaria tenuiventris	0	<1	<1	2	2	2
Phobocampe spp.[1]	2	7	13	11	6	5
Hyposoter tricoloripes	0	0	0	<1	<1	0
Parasetigena silvestris	0	<1	<1	11	25	23
Blepharipa pratensis	6	11	11	<1	4	<1
Tachinid sp.	0	0	0	0	<1	0
Hexamermis "albicans"	0	0	1	0	≤1	0
Total parasitized	9	19	26	32	59	62
Total hosts	1,117	1,200	1,400	260	600	270

[1]Includes *Phobocampe disparis* (Viereck) and a scarce undescribed species.

the population of *L. dispar*. The population at Würzburg collapsed to the point that further collections could not be made during 1975. It is believed that the high late-season parasitism by *A. melanoscelus, P. silvestris,* and (to a lesser extent) *P. disparis* contributed considerably to the collapse of this population.

Through the assistance of Mr. Hutchins, Agricultural Attaché, American Embassy, Warsaw, and Dr. Pieniazek, Director, Institute of Pomology, Skierniewice, the EPL was able to obtain space at the institute for rearing samples of gypsy moth larvae in 1975. Initial surveys were made by the senior author with the aid of Dr. Bakowski of the Pomology Institute. Unlike all other regions studied, no infestations were found in forest areas. The few foci of the gypsy moth found were either on plantings of mature linden trees (*Tilia*) along secondary roads or in apple orchards. Because the routine spray program eliminated the orchard populations, it was possible to study only the populations on linden. Two such infestations were found: one at Nieborów, a village about 15 km north of Skierniewice, and another at Alexandrowa, about 20 km west of Gostyn in west central Poland. Collections at Nieborów were made weekly May

6–July 10, 1975. Percent of parasitism in the 14 collections (4,618 larvae) appears in table 6.1–10.

The most important parasite was *A. melanoscelus,* which parasitized 9.8 percent of all larvae collected. Parasitism by the first brood of this braconid peaked at 12–15 percent during late May and early June, and by the second brood, at about 15 percent from June 16 to 19. The tachinid *Blondelia nigripes* (Fallén) was the most common dipterous parasite and attacked 4.7 percent of all larvae collected. The data in table 6.1–10 indicate that this parasite had one generation on gypsy moth and that peak parasitism (10–13 percent) occurred in mid-June. Poland is the only region in Europe where significant percentages of gypsy moth parasitized by *B. nigripes* were found. The most effective parasite late in the season was *P. silvestris,* which parasitized about 12 percent of the larvae collected in late June and early July (table 6.1–10). Although few larvae were parasitized by *B. pratensis,* parasitism of pupae by this parasite was much higher, showing that its impact on the population of *L. dispar* was greater than the data in table 6.1–10 indicate. Mean total parasitism at Nieborów was 19.8 percent. Because the infestation at Alexandrowa was not found until May 27 and, because of the distance, was sampled only

Table 6.1-10.—*Parasitism of larvae of* Lymantria dispar (L.) *at Nieborów, Poland, 1975*

Parasite species	Date collected and percent parasitized													
	May 6	May 12	May 19	May 23	May 28	June 2	June 9	June 16	June 19	June 23	June 27	June 30	July 3	July 10
A. porthetriae	0	0	0.2	0.4	0.4	0	0	0.2	0	0	0	0	0	0
A. melanoscelus	0	1.0	8.2	9.2	12.5	15.2	9.0	15.3	15.5	13.3	5.2	8.0	1.5	0
A. liparidis	0	0	0	0	0	0	.2	0	0	0	0	0	0	0
M. pulchricornis	0	0	0	0	.9	0	.2	.5	.2	0	0	0	0	0
Phobocampe spp.[1]	0	.5	.9	.6	.9	.4	.4	.5	.5	0	0	0	0	0
H. tricoloripes	0	0	.2	0	.7	.6	.7	1.3	.2	0	0	0	0	0
C. separata	0	0	0	.2	.2	.6	.7	0	4.5	4.8	4.8	2.0	1.5	0
B. nigripes	0	0	0	.6	2.6	6.2	6.9	10.0	12.8	8.5	3.0	1.0	3.0	1.0
P. silvestris	0	0	.4	0	0	.7	0	.5	.5	3.2	13	8.5	10	15
B. pratensis	0	0	0	0	0	0	0	.2	.2	.2	1.3	.5	5.0	0
Total parasitism	0	1.5	9.9	11.0	18.2	23.7	18.1	28.5	34.5	30.0	27.4	20.0	20.0	16.0
Total hosts	190	183	423	469	423	420	420	400	400	400	230	200	200	200

[1]Includes *Phobocampe disparis* (Viereck) and a scarce undescribed species.

every 2 weeks, the tabulated data will not be presented here. Total parasitism was 5–8 percent ($\bar{x}=7$ percent), much lower than at Nieborów, but the same two species of parasites, *A. melanoscelus* and *B. nigripes*, were dominant, mean parasitism by each being 3.4 percent and 2.1 percent, respectively.

Although 10 species of parasites were recovered at Nieborów (table 6.1-10), only four species (*A. melanoscelus, B. nigripes, P. silvestris,* and *B. pratensis*) were recovered at Alexandrowa. Although both the Nieborów and Alexandrowa infestations occurred on mature roadside linden trees, the Alexandrowa site was much farther from any heavily forested area that could have served as a reservoir for the host and its parasites. This could explain why the overall parasitism and species diversity of parasites at Alexandrowa were considerably less than that at Nieborów. Pawlowicz (1936) recorded *A. melanoscelus* (as *solitarius*), *A. porthetriae* (as *vitripennis*), *Exorista larvarum* (L.), *B. nigripes, C. separata,* and *B. pratensis* as larval or larval-pupal parasites of *L. dispar* in Poland, and also concluded that *A. melanoscelus* was the most important parasite. Although several species were recovered that

Pawlowicz did not find (table 6.1-10), no *E. larvarum* were recovered.

Studies on larval parasites of gypsy moth in Iran were made during 1976. Although suitable host plants were found in several different regions of Iran, the gypsy moth was found only in a crescent-shaped zone adjacent to the southern shores of the Caspian Sea extending from Tabriz in the west to the Elbrouz Mountains in the south and to Bojnurd in the east. In general, populations of *L. dispar* were higher in the humid zone of the coastal plain or in the hills immediately above the plain, where forest stands were composed of only two or three species, than at higher elevations where the tree species were mixed. Four stations were selected in the vicinity of Bandar Pahlevi, where collections were made periodically: A stand of *Alnus* and *Populus* planted between the rice paddies of the coastal plain; a grove of *Prunus* adjacent to the rice paddies; an open stand of *Gleditsia* located behind the coastal sand dunes near Amol; and a natural mixed stand of *Quercus, Alnus, Carpinus,* and *Gleditsia* in the hills above the coastal plain near Amol.

A total of 15,809 larvae was collected during the

season at the four stations. Of these, 1,046 larvae were parasitized, giving an overall apparent parasitism of about 7 percent. The actual parasitism was much higher but was masked by a very high mortality (85 percent) caused by pathogens, notably the protozoan *Nosema lymantriae* Weiser, and a nucleopolyhedrosis virus. Ultimately, larval survival approximated only 8 percent. Table 6.1-11 shows that the percentages of the larvae dying from disease were very high (>90 percent) early in the season and decreased with time beginning in early June. Coincident with this decrease in incidence of disease was an increase in total parasitism; therefore, total mortality did not decrease as sharply as incidence of disease (table 6.1-11). The two most significant groups of parasites were *Apanteles* spp. (dominant species *melanoscelus*) and several species of tachinids. Parasitism by *Apanteles* spp. was very low (<1 percent) early in the season, but increased steadily thereafter, reaching a peak of 12 percent during the week of June 4–10 (table 6.1-11). The effectiveness of *Apanteles* spp. was greatest at the *Prunus* orchard near Bandar Pahlevi, where parasitism by this genus peaked at about 25 percent on June 3. After mid-June, parasitism by *Apanteles* dropped sharply, but parasitism by tachinids increased, reaching a peak of about 11 percent during the week of June 18–24 (table 6.1-11). Parasitism by Tachinidae was highest at the *Alnus-Populus* stand near Bandar Pahlevi, where it peaked at about 17 percent on June 19.

A list of the larval parasites recovered in Iran appears in table 6.1-12, where it can be compared with the recoveries made in Europe (tables 6.1-5 to 6.1-10). Although Iran is a great distance from Europe, many of the same species were recovered in both places. One notable exception was the tachinid *Exorista larvarum* (L.), which was recovered in Iran, but not in Europe (table 6.1-12). However, *E. larvarum* has been reported from Europe by numerous other investigators (Burgess and Crossman 1929, Herting 1960a, Pawlowicz 1936, and Sisojević 1955). In general, the structure of the larval parasite complex in Iran was similar to that in Europe. The biggest difference between the two regions was the low apparent larval parasitism, about 7 percent, in Iran.

Parasitism in Europe is generally considerably higher (table 6.1-10). The low parasitism observed in Iran was in large measure due to the very high incidence of disease (table 6.1-11), the effectiveness of which is probably enhanced by the warm, humid climate in that region. It is perhaps noteworthy that about 44 percent of those larvae reared that did not die of disease were killed by parasites.

Pupal Parasites

Observations on pupal parasites of gypsy moth were made in southeastern France, Corsica, eastern Austria, Poland, and Iran.

Pupae of *L. dispar* were collected by handpicking and by looking under the bands used to attract larvae. In most localities, late-season populations of gypsy moth were so low that it was impossible to obtain sufficient pupae to make a realistic assessment of percentage parasitism by species. A similar problem arose in some high host-density localities, where it was very difficult to find pupae not attacked by virus.

Pupal collections were made in southeastern France during June 26–July 11, 1973. In general, gypsy moth pupae were very difficult to find except at a few localities, and only five samples, taken at three localities, contained over 25 pupae. Table 6.1-13 shows that *B. pratensis* was the dominant parasite emerging from host pupae at all three localities. This species is actually a larval/pupal parasite, and mean parasitism in collections of pupae was comparable to that in samples of late-instar larvae (tables 6.1-5 and 6.1-13). Parasitism by the chalcidid *Brachymeria intermedia* (Nees) was rather low, and only a very few pupae were attacked by an ichneumonid *Coccygomimus* sp. and the tachinid *P. silvestris* (table 6.1-13).

Hyperparasites were recovered only at Le Luc, where the host population was high (table 6.1-13). Data obtained there indicate that attack by hyperparasites tends to increase as the season progresses (table 6.1-13). Although *Brachymeria compsilurae* (Crawford) was the most abundant hyperparasite, a few specimens of *Dibrachys cavus* (Walker) were also recovered. The sarcophagid *Agria affinis* (Fallén) was recovered from 5 to 6 percent of

Table 6.1-11—*Parasitism and disease in larvae and pupae of*Lymantria dispar(L.) *in Iran, 1976*

Parasite species	Date collected and percent parasitized or diseased									
	Apr. 23–Apr. 29	Apr. 30–May 6	May 7–May 13	May 14–May 20	May 21–May 27	May 28–June 3	June 4–June 10	June 11–June 17	June 18–June 24	July 27
Apanteles spp.[1]	0.3	0.9	2.9	2.2	3.4	5.7	12.0	9.8	3.3	0
Meteorus pulchricornis	0	.1	.3	0	<.1	0	0	0	0	0
Casinaria sp.	0	0	0	<.1	0	0	0	0	0	0
Phobocampe n. sp.	0	<.1	.3	.2	<.1	0	0	0	0	0
Ichneumonidae spp.[2]	0	0	0	0	0	0	<.1	.2	<.1	.2
Tachinidae spp.[3]	0	0	<.1	0	.2	.6	4.5	1.3	11.4	5.7
Total parasitism	.3	1.1	3.5	2.4	3.7	6.3	16.6	11.4	14.7	5.9
Pathogens[4]	96.0	91.7	95.0	96.3	95.5	90.0	82.0	79.5	58.0	59.3
Total mortality	96.3	92.8	98.5	98.7	99.2	96.3	98.6	90.9	72.7	64.2
Host collected	1,955	1,508	1,560	1,553	2,089	1,829	1,923	528	2,164	700

[1]Includes *Apanteles lacteicolor* Viereck, *A. liparidis, A. melanoscelus* (dominant species), and *Apanteles* sp.

[2]Includes 2 pupal parasites, *Coccygomimus instigator* (F.) and *Lymantrichneumon disparis* (Poda).

[3]Includes at least 3 species, *Carcelia separata, Compsilura concinnata,* and *Exorista larvarum.*

[4]Includes nucleopolyhedrosis virus and *Nosema lymantriae* Weiser.

Table 6.1-12.—*Relative importance of gypsy moth parasites recovered in Europe and Iran, 1972-76*

Host stage(s) attacked	Family and species	Relative importance of parasite by region[1]						
		Southern France	Central France	Eastern Austria		Northern Bavaria	Poland	Iran
				1974	1975			
Egg	Eupelmidae: *Anastatus disparis* Ruschka	++++	—	++++	+++	—	0	+
	Encyrtidae: *Ooencyrtus kuvanae* (Howard)	++	—	+	0	—	0	0
Larva	*Braconidae:*							
	Apanteles lacteicolor Viereck	0	0	0	0	0	0	+
	Apanteles liparidis (Bouche)	++	+	++	+++	0	+	+
	Apanteles melanoscelus (Ratzeburg)	++	++	++	++	+++	+++	++
	Apanteles porthetriae Muesebeck	+++	+	+	+	+	+	0
	Apanteles sp.	0	0	0	0	0	0	+
	Meteorus pulchricornis (Wesmael)	++	+	++	+	+	+	+
	Meteorus versicolor (Wesmael)	+	0	0	0	0	0	0

Table 6.1-12.—Cont.

| Host stage(s) attacked | Family and species | Relative importance of parasite by region[1] | | | | | | |
| | | Southern France | Central France | Eastern Austria | | Northern Bavaria | Poland | Iran |
				1974	1975			
	Ichneumonidae:							
	Casinaria spp.[2]	+	+	+	0	+	0	+
	Phobocampe disparis (Viereck)	+	+++	++	++	+++	+	0
	Phobocampe spp.	+	+	+	+	+	+	+
	Hyposoter tricoloripes (Viereck)	+	++++	+	+	+	+	0
	Tachinidae:							
	Blepharipa pratensis (Meigen)	++++	++	++++	++	+++	+/++++	?
	Blondelia nigripes (Fallen)	+	+	+	0	+	+++	0
	Carcelia separata (Rondani)	++	+	+	0	0	+	+/++
	Compsilura concinnata (Meigen)	+	+	+	0	+	0	+/++
	Exorista larvarum (L.)	0	0	0	0	0	0	+/++
	Palexorista inconspicua (Meigen)	+++	0	0	0	0	0	0
	Parasetigena silvestris (R.-D.)	++	++	++++	++++	+++	++	?
	Peribaea tibialis (R.-D.)	0	+	+	0	0	0	0
	Siphona samarensis (Villeneuve)	0	0	+	+	0	0	0
	Mermithidae: Hexamermis "albicans (Siebold)"	0	0	+	++	+	0	0
Pupae[3]	Chalcididae: Brachymeria intermedia (Nees)	++[4]	-	0	—	—	0	0
	Ichneumonidae:							
	Coccygomimus instigator (F.)	0	—	0	—	—	++	+
	Coccygomimus sp.	+	—	0	—	—	0	0
	Lymantrichneumon disparis (Poda)	0	—	0	—	—	0	+

[1]0 = absent; + = mean parasitism <1%; ++ = mean parasitism 1%-5%; +++ = mean parasitism 6%-10%; ++++ = mean parasitism >10%; — = appropriate host stage not sampled; ? = lack of adult emergence from puparia collected prevented accurate determination of presence of these species.

[2]Includes Casinaria tenuiventris (Gavenhorst) and an undescribed species.

[3]The larval-pupal tachinid parasite Blepharipa pratensis is treated here only as a larval parasite.

[4]Parasitism >20 percent in Corsica.

Table 6.1-13.—*Parasitism of pupae of* Lymantria dispar (L.) *in southern France, 1973*

Locality and department	Date of collection	Number of pupae	Percent of parasitism by:					Total percent parasitism[1]
			Blepharipa pratensis	*Parasetigena silvestris*	*Brachymeria intermedia*	*Coccygomimus* sp.	Hyper-parasites	
Le Luc (Var)	June 27	58	22	0	2	0	0	24
	July 3	450	54	0	4	0	4	61
	July 6	500	28	0	2	<1	10	41
Subtotal		1,008	39	0	<1	<1	7	49
Brignoles (Var)	June 26	58	28	7	0	0	0	35
Le-Puy-Ste-Répa-rade (Bouches-du-Rhône)	July 11	33	6	0	3	0	0	9
Total		1,099	37.6	0.4	2.7	.3	6	47

[1]Totals may not add because of rounding.

the host pupae collected at Le Luc. Because it is likely that this species is a saprophage that merely feeds on diseased pupae of gypsy moth (Vasić and Sisojević 1958), it has been omitted from the tabulated data. Nevertheless, it is clear that significant percentages of pupae were attacked by parasites in four of the five samples taken.

Through the courtesy of Drs. Hurpin and Burgerjon of the Institut National de la Recherche Agronomique at La Minière, France, 328 pupae of *L. dispar* were received that had been collected in a cork-oak forest in Corsica during mid-June 1974. The percent of parasitism of the pupae was as follows:

Brachymeria intermedia	24%
Blepharipa pratensis	22
Coccygomimus sp.	1
Total	47%

In addition, about 6 percent of the pupae contained the sarcophagid *A. affinis*. Although the same parasites were recovered in southeastern France during 1973, parasitism by *B. intermedia* was much higher in Corsica. Because *B. intermedia* appears to prefer open sunny areas (Hoy 1976, Tigner 1974a), that species was probably favored in cork-oak forests, which tend to be rather open. Although *B. pratensis* was clearly the dominant parasite in southeastern France, it was more or less codominant with *B. inter-*

media in Corsica. Total parasitism (47 percent) was comparable to that observed in southeastern France (table 6.1-13).

In eastern Austria, only one locality was found where pupae could be collected. A single collection of 250 pupae was made at Trausdorf (Burgenland) on July 16, 1974. Only the larval-pupal parasite *B. pratensis* was recovered from this sample; however, that species had parasitized about 45 percent of the pupae. Jahn and Sinreich (1957) recorded *B. intermedia* and the facultative hyperparasite *Monodontomerus aereus* Walker as pupal parasites of *L. dispar* in eastern Austria.

Pupal collections were made in Poland during mid-July 1975 at the same two sites where the larval parasites were studied. Two samples totaling 518 pupae were taken at Nieboŕow, and one sample of 150 pupae at Alexandrowa. Percent of parasitism by localities was as follows:

	Nieboŕow	Alexandrowa
Blondelia nigripes	<1%	0%
Parasetigena silvestris	2	2
Blepharipa pratensis	12	19
Coccygomimus instigator	4	1
Total	18%	23%

At both localities, *B. pratensis* was clearly the most important parasite of pupae. The larval parasites *B. nigripes* and *P. silvestris* emerged from a few host pupae. The only true pupal parasite recovered was *Coccygomimus instigator* (F.), which was not abundant at either locality. Although no *B. intermedia* emerged from these samples, an adult resembling that species was observed at Nieborow. Pawlowicz (1936) recorded *C. instigator, B. pratensis, C. separata,* and *Exorista larvarum* as pupal parasites of gypsy moth in Poland. *Carcelia separata* was recovered from larvae of gypsy moth in Poland in 1975 but not from pupae. No *E. larvarum* was found during the 1975 studies in Poland.

In Iran, samples totaling 700 pupae of *L. dispar* were taken at five localities in the vicinity of Bandar Pahlevi on June 27, 1976. Total parasitism was rather low—5.9 percent, of which 5.7 percent was by Tachinidae (unidentified) and 0.2 percent by *Coccygomimus instigator.* In addition, one adult *Lymantrichneumon disparis* (Poda) emerged from a mixed sample of larvae and pupae collected near Bandar Pahlevi on June 19, 1976. This species was listed as a pupal parasite of *L. dispar* in Yugoslavia by Vasić and Sisojević (1958).

In four of the five regions where pupal collections were made, the vast majority of parasitized pupae were attacked by Tachinidae, primarily *B. pratensis,* which are actually larval-pupal parasites. Only in one single sample from Corsica did the hymenopterous parasite *B. intermedia* appear to be a dominant mortality agent. In general, Ichneumonidae (*Coccygomimus* spp. and *L. disparis*) did not appear to be important parasites of pupae.

Summary

A list of those parasites found during the studies in Europe and Iran appears in table 6.1-12. Only species that definitely developed from *L. dispar* are included. In addition, the relative importance of each parasite in each of the six regions covered has been rated.

Only two parasites of importance were positively reared from eggs of gypsy moth, *A. disparis* and *O. kuvanae,* both of which are established in North America (Burgess and Crossman 1929). Although several Scelionidae were recovered in very low numbers from egg masses, it was not positively established that they actually had developed on eggs of *L. dispar.*

A total of 547 samples (105,779 living larvae) of *L. dispar* was taken at 87 localities in the six regions explored. Table 6.1-12 shows that 25 species of larval or larval-pupal parasites were found attacking larvae of *L. dispar* in the six regions surveyed. Based on their relative importance (table 6.1-12), these larval parasites can be classified into four categories:

(1) The first category of larval parasites includes those species that consistently attacked significant percentages of larvae throughout Europe: *A. melanoscelus, B. pratensis,* and *P. silvestris.* All three of these species are established in North America.

(2) The second category of larval parasites includes those species that attacked significant percentages of larvae in certain regions or at certain localities within a region(s) but that were rare or not even recovered elsewhere: *A. liparidis, A. porthetriae, M. pulchricornis, P. disparis, H. tricoloripes, B. nigripes, C. separata,* and *P. inconspicua.* For example, *A. liparidis* was locally abundant in southern France during 1972–73 and in eastern Austria during 1974 and was the dominant hymenopterous parasite in the latter area in 1975; however, no hosts parasitized by that species were found in northern Bavaria, and only one was found in Poland. Similarly, *A. porthetriae* was clearly the dominant parasite of small larvae in southern France (table 6.1-5) but was rare or not recovered elsewhere (table 6.1-12). Like examples can be shown for the other species in this category. For the most part, these species seem to be well adapted to certain climates and forest types where they may equal or sometimes even surpass in effectiveness those species in the first category. With the exception of *P. disparis,* none of these species is established in North America (Hoy 1976), and all require alternate hosts (Burgess and Crossman 1929, Hoy 1976, Sabrosky and Reardon 1976). The alternate host requirement may ultimately preclude the successful establishment of these species in North America; this is an

unfortunate circumstance, because their absence from the North American fauna probably creates a number of refuges for the host larvae, which consequently escape attack by existing natural enemies (native and introduced). This is especially true for *A. porthetriae, A. liparidis,* and *H. tricoloripes,* which were found sometimes to parasitize significant percentages of larvae at low host densities.

(3) This category includes those species that did not appear to be effective parasties of gypsy moth in our studies but that have been cited by other workers as being important: *C. concinnata, E. larvarum,* and *Hexamermis "albicans".* In spite of the fact that *C. concinnata* was recovered very rarely, and that Dowden (1962) noted that the same species did not appear to be very important in central Europe during the 1920's and 1930's, Herting (1960a) ranked it as the fourth most effective tachinid parasite of the gypsy moth in Europe. Similarly, although *E. larvarum* was recovered in Iran but not in Europe, Herting ranked this species as the second most effective tachinid parasite in Europe. These differing observations on the effectiveness of these two tachinids may be explained by referring to the work of Sisojević (1959), who made long-term observations of tachinid parasites of the gypsy moth in Yugoslavia during an outbreak from 1954 to 1958. She found that each year thereof was marked by a pronounced dominance of one of the four most important tachinid species in the following order: *C. concinnata, E. larvarum, B. pratensis,* and *P. silvestris.* As tachinid dominance apparently changes with time, and *B. pratensis* or *P. silvestris* was the dominant species at the study sites, it is not surprising that *C. concinnata* was relatively rare. Both *C. concinnata* and *E. larvarum* are established in North America, although only the former appears to be a significant parasite there (Hoy 1976). Although the parasitic nematode *H. "albicans"* was recovered only in eastern Austria and northern Bavaria, where it did not appear to be an effective parasite (table 6.1-12), Artyukhovskii (1953) reported it as causing 60 percent mortality of larvae of *L. dispar* on a timber farm in the Soviet Union.

(4) The fourth category of larval parasites includes those species that never attacked significant percen-

tages of gypsy moth and have not been cited by other workers as effective parasites thereof: *Apanteles lacteicolor, Apanteles* sp., *M. versicolor, Casinaria* spp., *Phobocampe* sp., *Peribaea tibialis,* and *Siphona samarensis.* Many of these were recovered only once or twice from one or two regions (table 6.1-12). Several of these are known to be primarily parasites of other Lepidoptera: *A. lacteicolor* on browntail moth and *M. versicolor* on browntail and satin moths (Burgess and Crossman 1929). The limited recoveries of *P. tibialis* and *S. samarensis* from *L. dispar* constituted new host records (Sabrosky and Reardon 1976); therefore, it is believed that these species should be considered incidental parasites of gypsy moth and that they offer no potential for biological control efforts in the United States.

Although several true pupal parasites of gypsy moth were recovered in the studies, only *B. intermedia,* a chalcidid already established in the United States, appeared to be of any significance (table 6.1-12). In most localities, the larval-pupal parasite *B. pratensis* (also established in the United States) was the dominant parasite emerging from pupae. None of the ichneumonids ever attacked significant percentages of pupae (table 6.1-12). These results are very similar to those obtained by Vasić and Sisojević (1958) in Yugoslavia.

Recommendations

In general, it appears that all the important egg and pupal parasites and about half the important larval parasites found in Europe are already established in North America. Although other larval parasites of importance were found that would be useful additions to the parasite complex existing in the United States, most of these probably will never become established there because of stringent alternate host requirements. In view of these considerations, the following recommendations are made:

(1) Several larval parasites were found that appear to be promising, at least in certain areas, but either they have not been released in sufficient quantity to become established or they have been released only

recently: *B. nigripes, H. tricoloripes,* and *H. "albicans".* It is recommended that additional releases of these species be made. In the event that laboratory rearing techniques cannot be developed for these species, direct releases in small numbers would be preferable to no releases at all.

(2) Although the lack of suitable alternate hosts is probably responsible for the failure of such species as *A. liparidis* and *A. porthetriae* to become established in spite of releases of large numbers (Hoy 1976), completely discontinuing releases of these species is not advocated. Additional releases should be made in peripheral areas as the gypsy moth expands its range in North America, for it is possible that suitable alternate hosts may exist in areas where gypsy moth does not yet occur. This is certainly a possibility in the case of *A. porthetriae,* which was found to be important in southeastern France (table 6.1–5) and is known to be an effective parasite in other countries of southern Europe such as Spain (Romanyk 1973) and Yugoslavia (Vasić 1957) but was scarce in northern and central Europe (table 6.1–12). Additional releases of *A. porthetriae* could thus be made as the gypsy moth disperses in a southerly direction.

(3) Because it is highly unlikely that any "new" effective parasite would be found during the course of additional or future exploration in Europe, exploration there should be discontinued. (Europe could remain a worthwhile source of parasites for implementing the two preceding recommendations, however.) Although the considerable volume of Russian economic literature on gypsy moth suggests that any parasites to be found in the Soviet Union would probably be no more effective than those found elsewhere in the Old World, exploration in the U.S.S.R. or in the People's Republic of China should be carried out if the opportunity arises.

Future Research

In general, with the exception of those recommendations made above, exploration and introduction should be phased out; work with parasites, however, should not be dropped. Parasitism plays an important role in the cyclic population dynamics of the gypsy moth, but many elements of actual mechanics are poorly understood. Therefore, studies with parasites should be reoriented as follows:

(1) As a starting point, more long-term studies on parasite succession similar to those of Sisojević (1959) in Yugoslavia should be made. Because the parasite complex in North America has lower species diversity than that in the Old World, considerable differences in succession might also exist. It is not impossible that detailed analysis of trends in parasitism could also reveal patterns that could be of use in forecasting population outbreaks or collapses.

(2) The effects of different forest management techniques on parasite effectiveness should be ascertained.

(3) Because mean parasitism was found to be generally lowest at those sites in Europe where populations of gypsy moth were extremely low, it appears that the technique developed by Maksimović et al. (1970) (which involves introducing host egg masses to maintain parasite populations when gypsy moth populations are endemic) merits additional trials. Indeed, such an approach is especially indicated in the United States, where the ratio of host specific to polyphagous larval parasites is higher than in Europe.

These recommendations are by no means exhaustive, but they do offer examples of the type of research that might ultimately increase the effectiveness of those parasites already established.

Explorations in Japan and Korea by the ARS Asian Parasite Laboratory: 1975–77
Paul W. Schaefer

Because of the expanded gypsy moth program and the needs for new exotic material, the Asian Parasite Laboratory (APL) was established in Sapporo, Hokkaido, Japan, on May 15, 1975. Northern Japan was selected because of the occurrence of gypsy moth there in recent years. Laboratory facilities constituted office space in a rented private residence and use of laboratory, library, and working space in the Government Forest Experiment Station, Hitsujigaoka, Sapporo, which is under the direction of the National

Ministry of Agriculture, Forestry and Fisheries. The generous use of these facilities is acknowledged. In the 1977 season, for additional rearing facilities, a class-room at the Hokkaido International School was used. In addition to the APL staff, assistance at various times was provided by R.W. Carlson, ARS Systematic Entomology Laboratory, in 1976; by F. Hérard and G. Mercadier, ARS European Parasite Laboratory, in 1977; by H-P. Lee, Dongguk University, Seoul; and by K. Kanamitsu, Tokyo University Forest, Seto.

Objectives have focused on studies of the gypsy moth and their natural enemies with every effort made to acquire sufficient beneficial material for shipment to ARS quarantine facilities at Newark, Del.

Since APL activities have focused on all natural enemies, nonparasitic organisms have been identified as contributing significantly to gypsy moth population mortality. A pentatomid predator, *Dinorhynchus dybowskyi* Jakovlev, has been encountered frequently and warrants mention as a candidate for introduction. In gypsy moth populations in central Honshu in 1977, a dramatic epizootic attributed to the fungus *Entomophthora aulicae* (Reich.) Sorok. caused high levels of mortality to late-stage gypsy moth larvae similar to a report by Aoki (1974). This disease needs further study and consideration for microbial control. Finally, in Hokkaido, gypsy moth eggs were frequently lost through predation by over-wintering birds. This fact has required protecting egg masses from predation by erecting wire guards to permit collection of the eggs in spring. Without protective guards, survival of eggs is greatest in those eggs covered by snow. Winter mortality from predation has made sampling of larval and pupal stages difficult in many locations. These factors have an indirect but significant bearing on host availability for assessment of parasitic species. They are recorded here as significant gypsy moth mortality factors in Japan.

During the past three seasons, general concepts have been acquired of the bionomics of the gypsy moth in Japan, especially in Hokkaido, and in Korea. Bioecological information involving the gypsy moth is presented here as a background to understanding the ecology of the natural enemies encountered, and incidentally, the potential exotic agents for introduction into the North American entomofauna.

The Gypsy Moth in the Orient

The distribution of *Lymantria dispar* (L.) extends across Asia into Europe in a nearly continuous band between 30° and 60° N latitude (Kozhanchikov 1950). Great genetic variability occurs over this range. With Europe as the origin of the gypsy moth in North America, it is understandable that significant bioecological differences occur between oriental gypsy moths and those introduced into North America. A brief review of these differences helps to place the gypsy moth and their natural enemies in proper ecological settings.

Surveys for egg parasites of gypsy moth have covered all major islands in the Japanese archipelago and the Korean peninsula. Analysis of nearly 2,000 egg masses from 47 collection sites in Japan and 12 in Korea revealed that egg parasites are not widely distributed but do occur in some areas (fig. 6.1-5). Egg parasites were entirely absent at most collection sites, but if present, they occurred at low densities. *O. kuvanae* was found in central Honshu and southward. At 14 sites where *O. kuvanae* was found, 43 percent of egg masses were attacked with 4.9 to 5.6 percent of the eggs parasitized. Although levels of parasitism by *O. kuvanae* in Japan fail to approach levels reported in North America or Europe—for example, 30–40 percent in Connecticut (Dowden 1961) —occasionally an individual egg mass reflected extraordinary levels of parasitism: for example, 14 percent at Hamamatsu; 17 percent at Kaisugai; and 55 percent at Sekigahara (these locations are identified in fig. 6.1-5). These individual masses were often disturbed mechanically so that more eggs than normal were exposed to ovipositing females. Other comparisons of effectiveness of *O. kuvanae* are tabulated by Anderson (1976).

O. kuvanae is reported hyperparasitic on *Apanteles melanoscelus* (Ratzeburg) in North America (Crossman 1925; Kamran 1977), but this behavior has not been found in Japan. *O. kuvanae* serves as a host to another encyrtid, *Tyndarichus navae* Howard (Tachikawa 1963), which was confirmed during
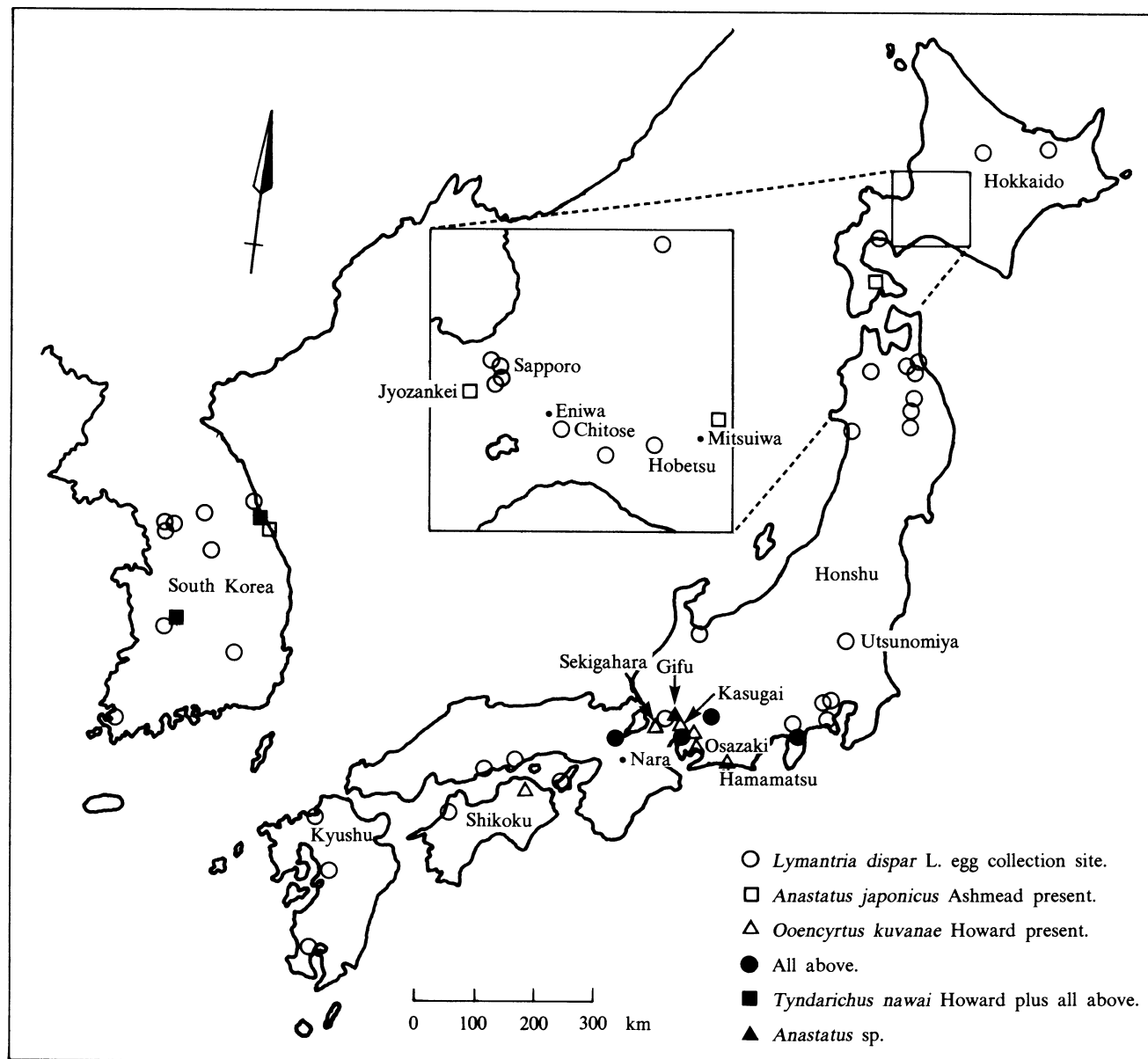
Figure 6.1-5.—*Collection localities for masses of*
Lymantria dispar *in Japan and Korea. Localities with*
egg parasites present are designated and towns named
in the text are identified.

recent rearings. *T. navae* appeared frequently in
Korea.

Anastatus japonicus occurs in Korea and Japan,
especially in Hokkaido, where it appears most suc-
cessful as a gypsy moth egg parasite. Although *A.*

japonicus is distributed throughout Japan, it is absent
from most collection sites (fig. 6.1-5). Preliminary
data suggest that *A. japonicus* was present in only
nine collection sites where 38 percent of 92 egg masses
were attacked. Egg parasitism was less than 4 percent

in Honshu, but at Jyozankei, Hokkaido, *A. japonicus* accounted for 6.8 percent mortality of eggs over two seasons. Comparable parasitism occurs in Korea. Some interesting biological differences emerge between areas sampled. In Hokkaido, *A. japonicus* overwinters in the host egg and approximately an equal ratio of sexes emerge in spring. In southern Honshu and Korea, data show that mostly males, with only an occasional female, emerge from gypsy moth eggs in spring. Only 12 females (1.4 percent) were found out of 826 *A. japonicus* adults that emerged from host eggs collected in Korea from December 1976 to April 1977.

It remains unclear where females are overwintering, but this may indicate that there are two biotypes of *A. japonicus,* or that *L. dispar* may represent an unnatural host for *A. japonicus* (see Hokyo et al. 1966, Kochetova 1968, Marovskaya 1973), or that improper synchrony of the parasite and host is forcing females to oviposit in older, well-developed host eggs, which results in male progeny (Bjegović 1964).

Anastatus japonicus in the eastern Orient seems less successful as a parasite of gypsy moth than in areas where it is exotic. In North America, parasitism reportedly reaches 20–30 percent (maximum recorded 33 percent) (Crossman 1925), and in Europe similar or higher levels are recorded (Krnjaic 1967, Kurir 1944, Stefanov and Keremidchiev 1961). In Japan, *A. japonicus* will attack host eggs in the genera *Dendrolimus, Malacosoma,* and *Nezara* (Hokyo et al. 1966), and in Russia, *Palomena prasina* (L.) (Marovskaya 1973). Surveys indicate that *A. japonicus* is no more effective with *Dendrolimus spectabilis* Butler (Hirose 1964; reported as *A. bifasciatus* in Hirose et al. 1968) than in gypsy moth.

One single male specimen of an *Anastatus* sp. (not *japonicus*) was recovered from a gypsy moth egg collected at Gifu, Honshu. This represents a new host record, but the exact identity remains pending.

Material collected previously by K. Kamijo, Hokkaido Forest Experiment Station, Bibai, revealed a *Telenomus* sp. (not *lymantriae* Kozlov (1967)) that was reared from eggs of *L. dispar* in Hokkaido. This *Telenomus* sp. has not been encountered in recent surveys and appears to be insignificant.

Most basic of comparative differences is female capacity for flight, size, and coloration. Females have the capacity of sustained or ascending flight, but it is uncertain where the transition occurs from flightlessness in Europe to flight capacity in Asia. Gypsy moths are larger in Asia than in North America (Leonard 1974) and show striking variation in both larvae and adults from various regions of Japan. Goldschmidt's culminating review of 25 years of study (1934) illustrates the various morphological variations and sex races, with partial genetic incompatibility, due to varying degrees of expression of intersexes when crosses are made between individuals from these different "sex races." Studies have confirmed this genetic variation. Females in certain crosses—for example, Hokkaido females and northern Honshu males—consistently produce all males in the F_1 generation, while the reciprocal crosses produce normal progeny of both sexes (Saito 1954). Phenotypic expressions show the great variability in color of adults throughout the Japanese archipelago and in Korea. Hokkaido males are generally lighter and resemble European and American specimens. In southern Japan, males are very dark brown in appearance. In Korea, male color is more chocolate brown. Females vary less throughout these regions but a distinctly darker form of female has been detected in Hokkaido. These appear in mid-September, and although taxonomists can see no differences in genitalia, this form may be temporally isolated from the more common form which appear as adults in early August.

Another behavioral difference between North American and Eurasian forms, as well as among Eurasian forms, is host-plant utilization. In northern Japan, periodic outbreaks occur in plantations of larch, *Larix leptolepis* (Sieb. & Zucc.) Gord., but larvae will feed on a large variety of tree species included in the genera *Quercus, Acer, Carpinus, Castanea, Salix, Prunus,* and others. In central Honshu and southward, the gypsy moth accepts these same hosts but frequently also defoliates persimmon, *Diospyros kaki* L.f. This is true also in China, where the moth is commonly referred to as the persimmon tussock moth. Despite these host preferences, the gypsy moth

remains a polyphagous opportunist and will accept a wide variety of host plants when more preferred species are lacking. Females in Japan are capable of flight and dispersal. In northern Japan they demonstrate a distinct preference for ovipositing on white birch (*Betula platyphylla* Sukatchev), even when white birch constitutes less than 30 percent of the forest composition (Schaefer 1978). In central Honshu, male flight activity occurs principally in the morning hours and ceases by early afternoon, whereas in Hokkaido the peak of flight activity occurs in the afternoon. Larvae show a feeding preference for larch in Hokkaido even though eggs are deposited on birch. Newly hatched larvae disperse from the birch trees to adjacent larch, which are eventually heavily defoliated, while the birches may be only slightly defoliated. Oaks, if present, are also favored as food.

Many taxonomists consider the Japanese gypsy moth a distinct subspecies, *L. d. japonica* (Motschulsky). Inoue (1957) recognized five subspecies in Japan, including one with males having nearly white hind wings. All these mentioned variations and differences in morphology, ecology, and behavior cause one to question the extent of conspecificity. Leonard (1974) has expressed similar thoughts.

Egg Parasites

Japan is the origin of *Ooencyrtus kuvanae* (Howard) and possibly *Anastatus disparis* Ruschka, both of which are established in North America; the first was subsequently established in southern Europe and North Africa. *Anastatus disparis* or *A. japonicus* Ashmead (there is need for taxonomic clarification here but the latter will be utilized hereafter) from Japan may have established in New England or at least contributed genetically, since live material from Japan was included among the material released (Crossman 1925). Interestingly, both these parasites tend to show greater success as egg parasites of gypsy moths in areas where they are exotic than in their native Japan.

Although records list additional egg parasites in Japan (for example *Pseudanastatus albitarsis* Ashmead and *Trichogramma dendrolimi* Matsumura

(Yasumatsu and Watanabe 1965)), recent surveys have failed to reconfirm the presence of these species.

Larval Parasites

Parasites of gypsy moth larvae contribute greatly to reductions in populations. If one thing is consistent, it is the inconsistency with which larval parasites appear from place to place or from season to season.

Apanteles liparidis (Bouché) is the most significant parasite throughout Japan. *A. liparidis* is important because it is gregarious and has high productivity; it averaged 11.3 and 41.7 cocoons per host larva in two respective generations in Hokkaido. The maximum recorded at APL has been 73 cocoons per late-stage host. Parasitism may be significant under favorable conditions; for example, at Jyozankei, Hokkaido, 49.2 percent was recorded in a collection made July 21, 1975. Cocoons in the field are heavily hyperparasitized.

Apanteles liparidis requires an overwintering lepidopterous larva as a host. This fact has prevented successful establishment in North America (Hoy 1976). In Japan it utilizes species of *Dendrolimus* that overwinter under bark scales or in the leaf litter. A new summer host, *Leucoma candida* Staudinger (Lymantriidae), was recorded; this species overwinters as larvae and may also serve as an overwintering host. *Euproctis* spp. are also candidates for alternate overwintering hosts for *A. liparidis*, although this has not been demonstrated.

Three additional *Apanteles* spp. are now recorded as gypsy moth parasites in Japan. In the 1977 season, *A. melanoscelus* (Ratz.) was first recorded from Hobetsu, Hokkaido, in parasite material sent to the United States for emergence. It does not appear to be a significant parasite in Japan. *A. melanoscelus* is a solitary parasite; it emerges from earlier stage hosts, and forms a whitish-yellow cocoon. It should not be confused with another new species of *Apanteles* that forms solitary, yellow cocoons and appears infrequently in Hokkaido; almost nothing is known about this parasite. A third new parasite, *Apanteles* new sp., with gregarious yellow cocoons, produces up to 17 (average 9.4, $n=20$) small, yellow cocoons per fourth-

stage host larva. Clusters of cocoons were positioned on the leaves, or very small twigs, quite unlike those of *A. liparidis* on the same trees, which were usually on the trunk or on the undersurface of larger limbs. This new parasite was encountered only at Utsunomiya, Tochigi Prefecture, Honshu, on May 30, 1977. Both new *Apanteles* spp. are being described by P. M. Marsh of the ARS Systematic Entomology Laboratory.

Other hymenopterous parasites appear occasionally. *Rogas lymantriae* Watanabe have emerged from fourth-stage hosts from a number of locations in Japan. The characteristic mummies formed in the cadaver of the host larva are easily recognizable; in the field these are very frequently hyperparasitized by *Eurytoma* sp. In experimental populations of gypsy moth at Eniwa, Hokkaido, *R. lymantriae* was responsible for 32.9 percent parasitism of third-stage gypsy moth larvae exposed June 21–July 1, 1976 (Yamaguchi 1977). Winter collections of *Rogas* mummies in larval *Orgyia recens approximans* Butler cadavers have tentatively been identified as *R. lymantriae*, indicating that the *Orgyia* is an overwintering host. The dissection of one host mummy revealed an apparently mature parasite larva, suggesting that this may be the overwintering stage of this parasite.

The ichneumonid, *Phobocampe* sp. (not *disparis* (Viereck)) (det. R.W. Carlson, Systematic Entomology Laboratory) has appeared at Utsunomiya, Honshu, and in several locations in Hokkaido. Parasitism is usually less than 2 percent. It appears that *P. "disparis"* in Japan and in Europe does not refer to the same insect on the basis of the above determination and on a marked difference in cocoon shape. This point needs clarification. Alternate hosts in Japan indicate *P. "disparis"* will also attack *Euproctis similis* (Fuessly) and *Malacosoma neustria testacea* Motschulsky (Yatsumatsu and Watanabe 1964).

The braconid, *Meterous japonicus* Ashmead (possibly = *M. pulchricornis* (Wesmael)) is another infrequently encountered parasite which usually accounts for less than 1 percent parasitism. Characteristic cocoons, which hang on silken threads are often hyperparasitized.

A significant complex of tachinids can be found in Japan or Korea, including the following species listed with relevant notes (nomenclature follows Sabrosky and Reardon 1976; an asterisk denotes previously confirmed host record in Japan):

Actia jocularis Mesnil—New Japan host record; genus not listed in Sabrosky and Reardon 1976.

Blepharipa schineri (Mesnil)—Record by association, confirmed in *Dendrolimus* sp.

**Blepharipa "sericariae"* of authors—Aoki (1969).

**Carcelia bombylans* Robineau-Desvoidy—Zwölfer (1972).

*Carcelia excisa (Fallén)—May refer to following species.

Carcelia separata (Rondani)—New determination.

**Compsilura concinnata* (Meigen).

**Exorista japonica* (Townsend)—Now in culture.

Exorista sorbillans (Wiedemann)—Record for Korea only.

Pales pavida (Meigen)—New Japan host record.

**Parasetigena silvestris* (Robineau-Desoidy).

Although there are 19 species discussed in Sabrosky and Reardon (1976) with distributions that very likely include Japan or Korea, definite host records for Japan include only eight or nine species, depending on some previous confusion between *C. excisa* and *C. separata*.

In Hokkaido, *P. silvestris* and *B. schineri* are larval parasites that emerge from late-star larvae. We have been unsuccessful in obtaining host-reared adults for species confirmation because of critical moisture needs in overwintering puparia. In nature, these puparia overwinter in the leaf litter, usually under heavy snow, until emerging as adults in spring. *P. silvestris* lays macrotype eggs on the dorsum of the host larva, while species of *Blepharipa* lay microtype eggs on foliage that is ingested by feeding larvae. *Blepharipa* and *P. silvestris* may sometimes account for 20–40 percent parasitism.

Nondiapausing tachinids include *A. jocularis Carcelia* spp., *C. concinnata*, *E. japonica*, and *P. pavida*. Adults of these are more readily obtainable because they emerge within the summer of pupation;

nevertheless only *E. japonica* has been cultured to date. Except for *E. japonica*, all evidence suggests that these species only occasionally contribute significantly to mortality of gypsy moth. *E. japonica*, which accounted for 9.1 percent parasitism at Okazaki, Honshu, in collections made June 18, 1977, is highly polyphagous; Yasumatsu and Watanabe (1964) list 24 recorded hosts. Females attach macrotype eggs to the integument of host larvae, and mature maggots emerge from the pupae. Little is known of the biological needs and behavior of this species.

A mermithid parasite, either *Hexamermis albicans* (Siebold) or a member of an *albicans* species-complex, has been found attacking gypsy moth larvae in certain areas in Hokkaido (Eniwa, Chitose, Hobetsu, and Mitsuiwa) but is absent at numerous other sites. Furuta and Koizumi (1975) reported this parasite previously but mistakenly identified it as a Gordioidea worm. They found it attacking about 20 percent of gypsy moth larvae exposed for prescribed periods during experimental studies on predation. In these continuing studies, *H. "albicans"* first appeared on July 7, 1975, from gypsy moth larvae exposed at Eniwa June 13–24, and accounted for 27.6 percent parasitism (Yamaguchi 1977). Similar efforts at the Asian Parasite Laboratory failed to recover a single nematode in 1976. Concurrent field collections of 843 gypsy moth larvae, mostly from Chitose, produced 310 juvenile nematodes for an average of 36.8 *H. "albicans"* per 100 hosts (not percentage parasitism due to unknown degree of multiple parasitism). These nematodes were recovered from hosts collected June 8–July 13, 1976.

In 1977, repeated exposure of host larvae at Chitose failed to yield any nematodes, but at Eniwa, recovery of 182 larvae on July 12 after exposure for 16 days produced 452 nematodes per host. This level of parasitism, estimated at 97 percent, was extraordinary but the method of exposure in the field was most natural because larvae were unconfined on saplings of *Salix* sp. about 2 m in height. Multiple and superparasitism occurred frequently; in one case, five nematodes and one unidentified tachinid emerged from one late-stage gypsy moth larva.

Little is known about the life cycle of this nematode. Rearing efforts are currently underway in the United States. Seasonal field work in Hokkaido is attempting to clarify questions concerning life cycle and voltinism, methods of attack on hosts by the unknown infective stages, reasons for the restricted and spotty distribution in Hokkaido, and necessary ecological information, especially on host range. To date, a sphingid, arctiid, and *Lymantria* spp. have been recorded as hosts in Japan. At Chitose, collections of gypsy moth larvae made in forests on a drier ridge and in a more moist river's edge produced greater number of *H. "albicans"* in the moist environment (0.5 vs. 41.5 and 77.6 *H. "albicans"* per 100 hosts). Although much more information is needed, this nematode is receiving continuing attention as a potential biological control agent.

Pupal Parasites

Although hymenopterous pupal parasites are usually found in low numbers, occasionally conditions are favorable for appreciable levels of parasitism. In many collection sites, the host population is so low or depleted by the time the pupal stage is reached that pupal material becomes exceedingly difficult to collect for assessment of parasitism. Parasitism by *Brachymeria lasus* (Walker) (=*obscurata* (Walker)) (det. A. Habu, National Institute of Agricultural Sciences, Tokyo) was first detected on June 3, 1977, when two females were captured ovipositing on newly transformed pupae of gypsy moth. The site, at Okazaki, Honshu, consisted of only several willow and persimmon trees in an open farmyard. The two females were returned to a field laboratory at Seto, where Dr. K. Kanamitsu (Tokyo University Forest) exposed fresh pupae to these females. Progeny were produced in 16 days at ambient laboratory temperatures. Subsequent pupal collections at Okazaki gave 41.6 percent parasitism by *B. lasus*. Adults pooled from these two sources were shipped and successfully cultured in the United States as discussed elsewhere in this chapter.

Although *B. lasus* is an occasional hyperparasite on both dipterous and hymenopterous primary parasites

(Joseph et al. 1973), our data failed to detect this behavior. In all field-collected host pupae yielding *B. lasus*, adults emerged directly from the host pupal case and subsequent dissection failed to reveal any evidence of hyperparasitic behavior indicated by remains of tachinid puparia. In one suspected case at Sekigahara, Honshu, a brachymerine emerged from a *Blepharipa* sp. puparia located within the host pupal case, an unusual pupation site for this parasite. This one specimen was later identified as *Brachymeria fiskei* (Crawford) (det. A. Habu). Both *B. lasus* and *B. fiskei* are sympatric in central Honshu and may be easily confused.

In Hokkaido, the ichneumonid *Coccygomimus disparis* (Viereck) (all ichneumonids det. R. W. Carlson, Systematic Entomology Laboratory) appeared at Mt. Teine, Sapporo, in 1976 in sufficient numbers to successfully ship live specimens and establish laboratory colonies in the United States. The gypsy moth outbreak caused defoliation in about 100 ha in 1976, and pupae were collected for an assessment of pupal parasitism. Of 746 pupae collected on July 30 and 813 collected August 5 and 6, only one and six (0.74 percent) *C. disparis* emerged, respectively. In the latter sample, three hyperparasitic *Theronia atalantae gestator* (Thunberg) also emerged and, assuming they were attacking the *C. disparis*, the maximum percent parasitism by *C. disparis* was thus 1.1 percent. Other ichneumonids collected to date include *Ephialtes capulifera* (Kriech.) from a gypsy moth pupa from Jyozankei, Hokkaido, collected July 21, and one unassociated *Coccygomimus instigator* (F.), collected in Sapporo in late August 1975.

In collections July 14, 1977, by personnel of the Government Forest Experiment Station, Sapporo, two males of *Lymantrichneumon disparis* (Poda) emerged from gypsy moth pupae for the first time. It consequently does not appear to be a particularly significant pupal parasite. This large ichneumonid normally overwinters as an adult (Carlson 1977).

Although more data are needed for low-density host populations, for reasons stated, little evidence is available to suggest that ichneumonid parasites are contributing significantly to pupal mortality in Hokkaido.

Prospective Biological Control Material

Viewed only from the standpoint of the field evidence of parasites in the Orient and their availability in terms of logistics and economics, an appraisal can be made of the parasites for potential introduction.

Apanteles liparidis has the greatest potential of all parasites because of its two generations in gypsy moth larvae, productivity per host insect, and dispersal capabilities. If suitable overwintering alternatives could be found in the United States, this species alone would be the most readily obtainable insect with the greatest potential as a successful biocontrol agent.

The newly discovered gregarious *Apanteles* sp. has many of the same attributes as *A. liparidis* but, unfortunately, may have similar overwintering requirements. Almost nothing is known of this new gypsy moth parasite.

Tachinid species in general rank a distant second. Although each species could be ranked according to effectiveness under field conditions and availability, there is little question that any one species would have a place in the complex of natural enemies in North America. In some cases, oriental species might compete with established exotics (for example, *Blepharipa* spp.), but it seems likely that the net detrimental effect on the gypsy moth will not be diminished but may be increased appreciably as a result of such competition. Much more bioecological information about each of these candidates is needed.

Brachymeria lasus warrants consideration as a potential agent for release. As a pupal parasite, it may compete with the established *B. intermedia* (Nees), which *B. lasus* resembles in all respects, except that *B. lasus* appears to be more polyphagous. It came from central Honshu and may be unsuited for use in the northernmost gypsy moth range but may be useful on other lepidopterous forest pests.

The nematode *Hexamermis "albicans"* is a potentially effective parasite. More information is needed on methods of attack, host range, and life cycle, but

use of such a novel exotic warrants serious consideration. Drawbacks such as limited dispersal capabilities, possible 2-year life cycle, and lack of host specificity must be considered.

Rearing, Evaluating, and Attempting to Establish Exotic Species and Redistributing Established Species

Attempts Before 1960

Richard C. Reardon

Impact of Native Species

In general, the native parasites did not have a significant impact on the gypsy moth as evidenced by the fact that, from 1900 to 1905, extensive damage resulted from the gypsy moth and the native enemies did not increase to any marked degree (Burgess and Baker 1938). Some of the native species recovered from gypsy moth at that time were two ichneumonids, *Coccygomimus pedalis* (Cresson), *Itoplectis conquisitor* (Say); and two tachinids, *Lespesia frenchii* (Williston) and *Exorista mella* (Walker).

Successful Establishment of Exotic Species and Redistribution of Established Species

The early importation effort (1905-14) was conducted enthusiastically, with entomologists located throughout Europe and Japan providing expert advice and cooperation. Unfortunately, numerous problems were encountered in shipping live host immatures and adult parasites and, therefore, in establishing laboratory colonies and providing adequate numbers for liberation. Also, published information concerning the parasites was scattered and unreliable. In spite of these difficulties, six of the species imported and introduced during this time became established (DeBach and Bartlett (1973) suggest that an insect is permanently established only if it is recovered in 3 successive years after release):

Two tachinids, *Compsilura concinnata* (Meigen) and *Blepharipa pratensis* (Meigen); one braconid, *Apanteles melanoscelus* (Ratzeburg); one ichneumonid, *Phobocampe disparis* (Viereck); one eupelmid, *Anastatus disparis* Ruschka; and one encyrtid, *Ooencyrtus kuvanae* (Howard) (table 6.1-14).

From 1914 until the early 1920's, the Massachusetts gypsy moth laboratory was engaged in assisting the spread of the established beneficial species and in determining their value as parasites of the gypsy moth (Crossman and Webber 1924). Several gypsy moth researchers suggested that, at that time, one of the major difficulties was the impossibility of securing certain parasites in adequate numbers for colonization under favorable conditions.

There was minimal activity involving parasite importation and distribution from 1915 through 1921, although activity resumed from 1922 through 1933. These importation and introduction efforts led to the definite establishment of two tachinids, *Parasetigena silvestris* (R.-D.) and *Exorista larvarum* (L.), and possible establishment of one chalcidid, *Brachymeria intermedia* (Nees) (table 6.1-15).

The following are brief reviews, pertinent to that time, of the species that became established between 1905 and 1960 (see Clausen 1956 and Dowden 1962 for extensive reviews).

Anastatus disparis.—The first few individuals liberated in New England (1908 and 1909) were adults from gypsy moth eggs collected in Japan, Hungary, and Russia, although all the colonizations after 1909 were obtained in New England. Since this species dispersed very slowly, approximately 65,644,193 were colonized through 1927. Parasitism averaged 11 percent from 1912 through 1927, although in some years the percentage parasitism of the small egg clusters was nearly twice as great as it was of the large clusters. Burgess and Crossman (1929) commented that "...*Anastatus* appears to be of more value in this country than in the countries from which it was originally obtained." This species became widely distributed but generally has a more northern range than *O. kuvanae*.

Table 6.1-14.—*Gypsy moth parasites successfully established in North America, 1905-14*

Species							
Current designation	Designation at time of importation	Country of origin	Stage of host parasitized	Date first liberated	Date of confirmed establishment	Overwintering stage and location	Remarks
Anastatus disparis Ruschka	*Anastatus bifasciatus* Fonscolmbe	Hungary and/ or Japan[1]	Egg	1908	1910	Full grown larva in gypsy moth eggs	Univoltine, host specific, females do *not* fly
Ooencyrtus kuvanae (Howard)	*Schedius kuvanae* (Howard)	Japan	Egg	1909	1910	Adult females	Multivoltine, host specific, females do fly, more southern range than *A. disparis*
Phobocampe disparis (Viereck)	*Hyposoter disparis* (Viereck)	Italy	Larva	1912	1913	Cocoon	Univoltine, heavy hyperparasitism of cocoon stage
Apanteles melanoscelus (Ratzeburg)	Same	Italy	Larva	1911	1912	Cocoon	Bivoltine, heavy hyperparasitism of cocoon stage
Compsilura concinnata (Meigen)	Same	Europe	Larva	1906	1909	Second stage maggot within hibernating host larva or cocoon	Multivoltine, (host range approx. 200 species), larviposits eggs in larva
Blepharipa pratensis (Meigen)	*Sturmia scutellata* R-D / *Blepharipoda scutellata* (R-D)	Europe	Larva	1908	1911	Puparium in soil	Univoltine, micro-type egg deposited on foliage

[1]Both Japanese and European stock released at the same colony site.

Table 6.1-15.—*Gypsy moth parasites successfully established in North America 1915-60*

Species							
Current designation	Designation at time of importation	Country of origin	Stage of host parasitized	Date first liberated	Date of confirmed establishment	Overwintering stage and location	Remarks
Parasetigena silvestris (Robineau-Devoidy)	*Phorocera agilis* of authors, not R-D / *Parasetigena agilis* of authors, not R-D	Europe	Larva	1910	1937	Puparium in soil	Univoltine, macro-type egg deposited on larva
Exorista larvarum (L.)	*Tachina larvarum* (L.)	Europe	Larva	1909	1940	Immature maggot within alternate hosts	Multivoltine, polyphagous macro-type egg deposited on larva
Brachymeria intermedia (Nees)	*Chalcis flavipes* Panzer	Europe	Pupa	1911	1942—leafroller 1965—gypsy moth	Adult females	Bivoltine

Ooencyrtus kuvanae.—This species was introduced from Japan and was later introduced and established in Europe. A total of 25,677,587 was colonized from 1909 through 1927. This species was bred at the laboratory and the adults liberated, while *Anastatus* was colonized as mature larvae within gypsy moth eggs. It became widely distributed over most of the infested part of New England but has not done well in the colder sections of the infested region (Bess 1961).

Phobocampe disparis.—A total of 12,543 was liberated in New England through 1927. This species has not been an important parasite of the gypsy moth, but parasitism was frequently much higher in dense woodland than along the wood's edge. This species is limited by heavy hyperparasitism of overwintering cocoons and mortality of parasite larvae within the host.

Apanteles melanoscelus.—A total of 155,653 was liberated from 1911 through 1927, although all the parasites liberated after 1913 were obtained from stock established in New England. This parasite often is very abundant in limited areas, and collections of gypsy moth larvae may show 20 to 30 percent parasitism. Its initial natural spread was in a northerly direction. The effectiveness of this species may be limited by hyperparasitism (95 percent) of the overwintering cocoons.

Compsilura concinnata.—A total of 147,759 was colonized from 1906 through 1927. This species naturally spread westward beyond the gypsy moth dispersion line (approximately 125 miles), which indicated that it would eventually inhabit a large part of the United States without further assistance. In a few instances parasitism as high as 80 percent was recorded, although this species has not done as well in the more xeric habitats in southern Massachusetts. Since its introduction this species has been reared from nearly 200 species of Lepidoptera; most of these records were reported by Webber and Schaffner (1926).

Blepharipa pratensis.—A total of 84,643 was colonized from 1908 through 1927, although those colonized since 1917 were recovered incidentally while making collections of gypsy moth larvae and pupae for other purposes. This species was of great importance in the lightly infested areas along the leading edge as well as in heavy infestations in previously infested areas. In general, average parasitism fluctuated from 5 percent to 25 percent.

Parasetigena silvestris.—A total of 231,086 foreign puparia was hibernated at Melrose from 1923 through 1927; because of extensive mortality, however, only 20,723 were liberated from 1910 through 1927. Definite establishment was not confirmed until 1937; by 1941, it had spread throughout most of the area infested by gypsy moth.

Exorista larvarum.—The total colonization of this species in New England from 1906 through 1927 was 42,152. Recovered for the first time in 1940, by 1941 this species was reared from gypsy moth larvae collected from Maine to Connecticut. This Palearctic species is exceedingly similar to the Nearctic *E. mella,* and probably misidentifications of both have been made (Sabrosky and Reardon 1976).

Brachymeria intermedia.—A total of 20,798 adults was liberated; most of these were colonized in 1911, but 644 were colonized in 1927. This species was not recovered from gypsy moth until the mid-1960's.

The benefits derived from several of these established species were not limited to parasitism of the gypsy moth, because they parasitized many native insects as well as other introduced European pests (for example, satin and browntail moths).

Unsuccessful Establishment of Species

There were approximately 16 species that were imported and liberated but that never became established: Three braconids (*Apanteles porthetriae* Muesebeck, *A. liparidis* (Bouche), *Meteorus pulchricornis* (Wesmael); 12 tachinids (*Palexorista gilva* (Hartig) probably *P. inconspicua*), *P. inconspicua* (Meigen), *Carcelia gnava* (Meigen), *Carcelia separata* (Rondani), *Exorista japonica* (Townsend), *E. segregata*

(Rondani), *Blondelia nigripes* (Fallen), *Zenillia libatrix* (Panzer), *Blepharipa sericariae* of authors, *Pales pavida* (Meigen), *Tachina magnicornis* (Zetterstedt) and *Blondelia piniariae* (Hartig); and one chalcidid (*Brachymeria obscurata* (Walker) probably *B. lasus*).

Most of these species were not established probably because of the absence of alternate hosts or unfavorable conditions at the time of colonization (Howard and Fiske 1911). Hoy (1976) also suggests reasons for nonestablishment of gypsy moth parasites in North America.

Attempts After 1960

Distribution and Attempted Establishment of Parasites

Jack R. Coulson

As has been discussed in previous sections of this compendium, the most recent period of importation of natural enemies of the gypsy moth began in 1963. As is the case with all shipments of biological control material received from foreign countries, the U.S. Department of Agriculture required shipments of gypsy moth material to be received in quarantine facilities. Material shipped to the United States from 1963 through 1973 was received at the ARS quarantine facility at Moorestown, N.J. From 1974, shipments were received at the newly built ARS Beneficial Insect Research Laboratory, Newark, Del.

A record of the species of gypsy moth natural enemies sent to ARS quarantine facilities since 1963 and of the species that have been cleared in and forwarded from quarantine is discussed elsewhere (see table 6.1–1).

Early Utilization of Imported Material

In 1961, the Plant Protection Division (PPD, now the Plant Protection and Quarantine Programs of the Animal and Plant Health Inspection Service, Department of Agriculture) of the Agricultural Research

Service (ARS) established a Gypsy Moth Methods Improvement Laboratory at Otis Air Force Base, Mass., to supplement research in improving and developing new control techniques for the gypsy moth. In addition, the Division of Plant Industry of the New Jersey Department of Agriculture (NJDA) became active in testing alternate methods of gypsy moth control, often in close cooperation with PPD. Beginning in 1963, some of the natural enemies found as a result of the early Public Law 480 projects were imported, via the quarantine facility of the ARS Beneficial Insects Research Laboratory at Moorestown, N.J. At first these insects were utilized by PPD and NJDA primarily in field-release programs. In 1966, however, the scope of the program at the PPD laboratory at Otis Air Force Base was broadened to permit culture of both domestically collected and exotic parasites, in order to obtain greater quantities of these natural enemies for release in gypsy moth infested areas of the Northeastern United States. To accomplish this goal, personnel at the Otis laboratory had to develop techniques necessary to culture the imported species (Reardon et al. 1973) and a new species was described (Reardon 1970). This program continued until the end of 1971.

Domestic Distribution of Imported Species, 1972–77

In late 1971, the Plant Protection and Quarantine Programs (PPQ) discontinued their parasite rearing activities at Otis Air Force Base, and cooperative agreements were initiated with several States to rear and distribute gypsy moth natural enemies. The principal organization involved in these cooperative activities with PPQ was the Division of Plant Industry, NJDA, to which all cultures then existing at Otis were given, although some culture activities in cooperation with PPQ were also conducted by the Maryland Department of Agriculture.

From 1972, most of the exotic material leaving ARS quarantine was sent to the NJDA laboratories at Trenton, which were charged under the PPQ

cooperative agreement with developing rearing techniques, large-scale culture of the material (if possible), and making these species available on request to other States for utilization in their own gypsy moth control programs or for further studies. Several other States also became involved in limited culture activities. A principal organization to do so was the Bureau of Forestry laboratory of the Pennsylvania Department of Environmental Resources at Harrisburg. Beginning in 1974, some of the imported material was shipped directly from ARS quarantine to this laboratory. Beginning in 1975, certain specifically requested exotic species were also made directly available by the ARS quarantine facility to research workers of the Connecticut Agricultural Experiment Station and to the Forest Service at the Northeastern Forest Experiment Station, Connecticut. Very little of the imported material was directly utilized by the ARS quarantine facility at Newark, Del., in field-release studies.

Release of some of this exotic material was made against the gypsy moth in the New England States, New York, New Jersey, Pennsylvania, Delaware, and Maryland. Releases of nonhost-specific parasites were also made against alternate hosts in noninfested States of Wisconsin, Indiana, Virginia, West Virginia, and North Carolina, as a result of the PPQ-NJDA cooperative agreement.

Establishment of Parasites

Although several of the newly imported natural enemy species were recovered at release sites during the year of release, very few field recoveries were made that gave any indication of permanent establishment in the United States. Two recent (1977) recoveries of interest are a *Meteorus* species, probably *pulchricornis*, from a malaise trap in Wisconsin in the year following its release in the area (Coppel 1978), and the recovery in the month of its release in Pennsylvania of an adult specimen of *Exorista segregata*, reared from a larval forest tent caterpillar, *Malacosoma disstria* Hübner (Ticehurst and Simons 1977). Neither of these

recoveries, of course, is a definite indication of permanent establishment.

Hoy (1976) presented a number of possible reasons for lack of establishment of the many gypsy moth parasites unsuccessfully introduced in past programs and in the early part of the current program. One suggestion was that inadequate numbers of the various species may have been involved in the release attempts. Data on individual species released in the current program (listed in later sections of this chapter) indicate that in most cases large numbers were released overall but that perhaps in some cases individual releases might have been larger.

Hoy also pointed out that earlier successful releases for the most part consisted of large quantities of field-collected noncultured material. Except for the early labor-intensive collections in Spain, most of the collections overseas consisted of small numbers. This was primarily because collections were made generally in lower host-population densities than those of earlier exploration programs, and because of a limitation in available manpower. Thus, a dependence on laboratory propagation was required in order to augment populations of the natural enemies for adequate releases. However, Hoy (1976) and others have also expressed concern over the possibility of genetic deterioration during laboratory propagation. It is possible that this has been a contributory factor in the apparent failure to effect establishments during the current program.

Hoy (1976) noted that most of the currently established gypsy moth parasites were established within 3 years of their initial release but also listed three exceptions: *Parasetigena silvestris* (specific to the gypsy moth) and *Exorista larvarum* and *Brachymeria intermedia* (both relatively polyphagous parasites). With the exception of the two *Apanteles* species, *liparidis* and *porthetriae*, which were unsuccessfully introduced in earlier programs and for which adequate overwintering hosts apparently do not exist in North America, the parasites released in large numbers during the current program were polyphagous species. It is conceivable that one or more of

these may yet prove to be established. Of course, the effectiveness of such establishments remains to be seen.

Most of the major natural enemies of the gypsy moth found during the past and current exploration programs have already been successfully established in the United States. As mentioned before, many of the parasites introduced during the recent importation program have been relatively minor parasites, highly polyphagous species, or species for which the gypsy moth itself serves as an alternate host, and the importance of their eventual establishment would appear to be in question. Another question to be answered is whether some of the species recently discovered in Asia, about which additional information is required, will prove to be effective gypsy moth parasites in the United States.

Quarantine Processing of Exotic Species and Distributions to Various Laboratories: 1961–77

Robert C. Hedlund, Lawrence R. Ertle, and Jack R. Coulson

As stated previously, most of the imported material, after elimination of any host material, secondary parasites, etc., was forwarded to other facilities for culture, further study, and field release. Most of these shipments were made to the APHIS Gypsy Moth Methods Improvement Laboratory at Otis Air Force Base, Mass., and the New Jersey Department of Agriculture biological control facilities (NJDA) at Trenton, from 1963 to 1971. From 1972, most of the shipments were sent to the NJDA, but from 1974 to 77, shipments were also made to facilities in Pennsylvania and Connecticut.

This section details the procedures used in clearing material through quarantine and the few studies of the exotic material conducted in quarantine by ARS personnel. Such studies have been limited and have been conducted only since the initiation of the Expanded Gypsy Moth Program in 1975, which provided funds for a research entomologist at

Newark, Del., to take part in the gypsy moth program.

Quarantine Facilities and Procedures

The 147.4 m^2 Quarantine Section of the Beneficial Insects Research Laboratory (BIRL) was constructed in 1973 on the University of Delaware Agriculture Farm to replace the aging facility at Moorestown, N.J., which had served as the biological control quarantine facility since the 1940's. The 12-room complex was specifically designed to act as a clearing house for the introduction of exotic parasites and predators. Consignments are also transshipped to other USDA facilities, State agriculture departments, and university facilities designed to handle pathogenic or phytophagous organisms.

The Quarantine Section is divided into three security zones, each with a specific set of guidelines. To enter, all authorized personnel, supplies, and shipments must pass through the Quarantine Office and the Packing & Shipping (P&S) areas; this first security zone acts as an anteroom that monitors the movement of material and personnel. A locked, magnetically sealed door leads from the P&S area through a three-cubicle serpentine entrance to the General Quarantine Laboratory (GQL). The GQL functions as an insect and equipment storage, recycling, and preparation area. A third, magnetically sealed door leads to the central corridor connecting the locked doors of the Foreign Package Handling (FPH) room and the Emergence, and Rearing rooms of the third security zone.

A foreign consignment received from the shipping agent is taken to the FPH room, which is designed to handle the preliminary processing of an incoming shipment. The individual packages are opened in a .3398-m^3 plexiglass isolation booth where the beneficial material is microscopically examined to remove diseased, deformed, or injured specimens, and the healthy specimens are identified and transferred to specially designed double-walled quarantine containers. The potential beneficial material is taken from the FPH room to the Emergence, Rearing (also used

for room-temperature adult storage), or GQL (adult, immature or overwintering cold storage) areas, or to the P&S area for transshipment to other quarantine facilities. The nonbeneficial materials of an incoming shipment (wrappings, package carton, insect or plant hosts, etc.) are placed in a plastic bag and sterilized in a steam autoclave for 90 minutes (30 psi, 110° C).

Specimens from each consignment are mounted or preserved in 95 percent ethyl alcohol and sent to the Systematic Entomology Laboratory (SEL), Beltsville, Md., to obtain accurate identifications or to confirm determinations by the BIRL Quarantine Officer. The release clearance of the potential beneficial organism is determined by reviewing available ecological and biological information based on taxonomic determinations and discussions with knowledgeable experts. The organism is assigned to one of four quarantine clearance categories:

1. The organism is unsuitable or too dangerous for continued experimentation. All material placed in this category is destroyed in Quarantine.

2. The organism is considered as a potential biological control agent, but ecological and biological data are missing, questionable, or need clarification. All this material is restricted to experimentation within a primary quarantine facility.

3. The organism is beneficial, but additional experimentation is necessary to determine its environmental impact. This material is given clearance for release to secondary quarantine facilities.

4. The organism is considered to be environmentally safe for general release. Parasites and predators placed in categories 3 and 4 are transferred to shipping containers and taken to the P&S area for documentation and shipment to secondary quarantine facilities for further observation or to cooperators for rearing and/or release.

Quarantine aspects of biological control importation programs are discussed in more detail by Fisher (1964).

Studies of Gypsy Moth Natural Enemies in Quarantine

The majority of exotic gypsy moth natural enemies received in quarantine have been cleared and sent to cooperators for rearing or release. All foreign species of predators received, with the exception of Calosoma sycophanta (L.), have been studied extensively in quarantine, particularly the tenebrionid beetle, Akis bacarozzo (Schrank), from Morocco and the stink bug, Dinorhynchus dybowskyi Jakovlev, from Japan. Only three general groups of gypsy moth parasites have been held for long periods in quarantine during the expanded program: The egg parasites Telenomus and Gryon species and Anastatus ?kashmirensis Mathur, and various tachinid puparia.

Telenomus and Gryon egg parasites were collected in Morocco and first shipped to the BIRL quarantine facility in December 1975. Two additional shipments were received in February 1976. Unfortunately, no fresh laboratory-reared egg masses were available for parasitization. Masses in which the eggs were fully embryonated were supplied, but no progeny emerged. The eggs that had been exposed to parasitization were refrigerated for 8 months in the event that the parasites were diapausing, but no progeny emerged. Because of the lack of suitable host material when these parasites were received, it cannot be concluded that they would not be effective parasites. Improved host culture methods will allow a more extensive and accurate evaluation if they are received again. Specific identification of Telenomus parasites are extremely difficult because of the general lack of up-to-date knowledge of the taxonomy of the group. The genus, which is of great importance in biological control, is badly in need of revision.

Several shipments of eggs masses of the Indian gypsy moth, Lymantria obfuscata (Walker), were received in quarantine during 1975 and 1976. As adults of Anastatus ?kashmirensis emerged, they were sent to the Forest Service, Northeastern Forest

Experiment Station, Hamden, Conn., for study and evaluation. It became apparent, through conversations between field researchers and SEL taxonomists, that the identity of this species was not certain, and its potential as an effective parasite was also in doubt. Studies were initiated in quarantine to answer these questions. Various cultures of *Anastatus* spp. are being maintained at BIRL. These include *A. ?kashmirensis* from India and *A. disparis* Ruschka strains from Austria, Iran, and Connecticut. In addition, parasitized gypsy moth egg masses have been collected from the *A. disparis* type locality in Hungary and from the *A. japonicus* Ashmead type locality at Atami, Japan. Both European and Japanese strains have earlier been released in the United States. When adults of these emerge, they will be sent to BIRL for biological and taxonomic evaluation. All possible cross-mating tests are being conducted among these cultures, and studies are being done of each culture to determine the fecundity, longevity, and parasitization effectiveness of the species. These parasites overwinter as mature larvae within the host eggs, and therefore the results of these tests cannot yet be tabulated. Results of cross mating between *A. ?kashmirensis* and *A. disparis* have not produced any female progeny, although male emergence has been common. This indicates that the females are not being fertilized and that *A. ?kashmirensis* is indeed a distinct species.

Tachinid puparia recovered from the gypsy moth have been held in quarantine until the adults emerged and are identified. Mortality has traditionally been very high for overwintering puparia. Tests have shown that if the puparia are kept in 100 percent relative humidity, from the time of arrival until the adults emerge, mortality is greatly reduced. This level of humidity has been maintained by storing the puparia in petri dishes enclosed in a plastic bag containing wet sponges or paper towels. If puparia are allowed to contact a moist surface such as filter paper, then mortality due to fungi increases. Puparia received from the Asian Parasite Laboratory (APL)

in 1976 were few in number and all were dead before overwintering was completed, except for eight adults that had been recovered from larvae of the silk moth, *Bombyx mori* L. These adults were shortlived and no feeding, mating, or oviposition occurred. Puparia received during 1977 have fared only slightly better. APL sent 946 puparia of *Blepharipa*, *Parasetigena*, and *Exorista*. These were immediately placed in high humidity. In October they were X-rayed at the Pennsylvania Bureau of Forestry laboratory at Harrisburg, and 421, or 45 percent, of these appeared to be viable. In May–June 1978, however, only 6 percent of the overwintering *Blepharipa* and *Parasetigena* puparia produced adults (Fuester 1978).

Gypsy Moth Parasite Distribution Program of the Animal and Plant Health Inspection Service/Plant Protection and Quarantine Programs
Richard C. Reardon

The Animal and Plant Health Inspection Service (APHIS) Gypsy Moth Parasite Distribution Program was initiated as the gypsy moth continued to spread through New York and into New Jersey and Pennsylvania. The appeal for parasites for these new areas was great, and Executive policy encouraged Plant Protection and Quarantine (PPQ) to establish Project-4 Cooperative programs (USDA) 1977). The program objective was "to establish known gypsy moth parasites and predators in all infested areas imminently threatened by the moth." Goals for achieving this objective were to release in newly infested areas those parasites and predators which are presently established in the Northeast; to release in all infested areas new exotic species as they become available; and to release polyphagous species in areas threatened by gypsy moth spread.

Two Project-4 Cooperative Agreements were initiated, one with the New Jersey Department of Agriculture (NJDA) and the other with the University of Maryland. The NJDA agreement, effective July

1971, provided for rearing and furnishing gypsy moth parasites for release in other infested states; rearing and releasing parasites in New Jersey and evaluating the results, and additionally developing mass rearing techniques for newly introduced parasites. The agreement was originally funded for $36,000 per year; this increased to $76,000 for both FY–72 and FY–73, $86,000 for FY–74, and $100,000 each for FY–75, FY–76, FY–77, and FY–78. The numbers of parasites distributed to various States from 1972 through 1977 are summarized in table 6.1–16.

The University of Maryland agreement was put into effect for FY–72 at $10,000 per year for rearing, releasing, and field evaluation of polyphagous parasites. After FY–72, the agreement was placed with the Maryland Department of Agriculture and continued unchanged until FY–77, when it was reduced to $2,500 per year, with funds to be applied to field evaluation of both established and exotic species of parasites.

In 1977, the Deputy Administrator for PPQ appointed a task force to evaluate the present program and to make recommendations on future actions. The major conclusions of the task force were that PPQ had not provided a formal structure to allow coordination and input among the many cooperating agencies; that only minimal documentation of results existed, and that done was not coordinated in such a fashion that results could be comparable among geographical areas. In fact, because of the poor quality and often complete lack of program evaluation, it would be difficult to justify this program at present if it were evaluated upon the presently available data. The task force made several recommendations to alleviate these problem areas: PPQ should assume a strong leadership role in the evaluation of data to document the results of the program; not use available resources for rearing, distribution and establishment of parasites in the periphery of infested areas or in advance of gypsy moth infestations except in a research support role; establish a steering committee composed of representatives of all participating agencies to furnish

guidance in the rearing, distribution and evaluation of parasites; identify research needs; select potential establishment sites; and provide assistance for the foreign exploration program.

The most urgent responsibility of PPQ is to evaluate the accomplishments of the Gypsy Moth Parasite Distribution Progam in terms of program objectives and overall APHIS policy. For example, the program objective "to establish parasites in all infested areas and in areas imminently threatened . . ." appears to be in direct conflict with the policy of the parent agency. In addition, although one could establish the value of the early introduction and establishment of exotic parasites, no documentation exists to justify work to establish parasites in peripheral gypsy moth populations or in areas where the gypsy moth might spread. Parasites by themselves will not prevent the spread of the gypsy moth, prevent outbreaks, or control high density gypsy moth populations. They do have a role in suppressing gypsy moth populations in the generally infested area along with other mortality factors, although at this time the contribution of parasites as a single mortality factor to the overall mortality complex is vague and somewhat contradictory. The data from the Project–4 Cooperative Agreements should be valuable in elucidating the role of parasites because this type of documentation has been scarce. Program documentation has come from tables containing numbers of parasite species released in various States (table 6.1–16). It is hoped that the task force recommendations will be implemented as part of the Parasite Distribution Program in subsequent years.

Notes on Species Cultured and/or Released, 1961-77

Tachinidae

Blondelia nigripes (Fallén)
Robert A. Fusco

Blondelia nigripes (Fallén), called *Lydella nigripes* in the early literature, is a multivoltine, solitary or

Table 6.1-16—*Gypsy moth parasite shipments under the APHIS-PPQ Program Cooperative Agreement with NJDA, 1971–1977*

	Apanteles liparidis	*A. melanoscelus*	*A. porthetriae*	*Meteorus Pulchricornis*	*Coccygomimus disparis* (India)	*C. disparis* (Japan)	*C. instigator*	*C. turionellae moraquesi*	*C. turionellae turionellae*	*Brachymeria intermedia*	*Blepharipa pratensis*	*Blondelia nigripes*	*Compsilura concinnata*	*Exorista larvarum*	*E. rossica*	*E. segregata*	*Palexorista sp.*	*P. inconspicua*	Totals
Del.	84,350	6,300	12,250	10,657	1,500	2,500	1,000	23,500	31,800	106,000	—	2,800	17,340	6,439	—	—	11,500	9,250	327,186
Ind.	—	—	—	—	—	3,400	—	—	—	15,350	—	500	822	—	—	—	—	8,250	28,322
Ill.	—	—	—	250	—	2,500	—	—	500	15,200	—	1,000	1,435	—	—	—	500	8,250	29,635
Ky.	—	—	—	250	—	—	—	—	—	—	—	—	—	—	—	—	4,000	—	4,250
Md.¹	96,250	—	15,650	12,330	16,914	2,000	6,500	—	6,000	51,000	62	1,200	—	8,200	—	—	11,000	17,250	244,356
N.Y.	8,700	400	1,250	2,926	750	500	750	750	3,750	2,000	—	750	—	1,450	1,000	1,000	3,500	2,000	31,476
N.C.	4,000	—	2,000	5,500	4,500	—	1,500	6,100	7,500	129,100	—	—	9,350	8,000	—	—	16,000	8,500	202,050
Pa.	9,000	12,600	7,500	7,270	10,342	—	3,250	—	25,000	833,100	—	—	—	10,550	6,100	4,600	13,750	11,500	954,562
R.I.	8,000	—	—	—	—	—	—	—	—	—	—	—	2,200	—	—	—	—	—	10,200
S.C.	—	—	—	—	—	—	—	—	—	1,750	—	—	—	—	—	—	—	—	1,750
Tenn.	—	—	—	500	—	—	—	—	—	—	—	—	—	—	—	—	1,000	1,000	2,500
Va.	4,100	500	1,600	2,634	3,000	2,500	500	500	4,500	52,500	—	4,400	—	150	—	—	2,000	1,750	80,634
W.Va.	12,000	—	1,950	1,479	1,000	1,500	100	—	—	4,000	—	2,000	500	—	—	—	—	7,000	31,529
Wis.	—	2,000	—	500	—	—	—	—	—	16,400	—	1,750	1,200	—	—	—	—	—	21,850
Mass.	2,000	—	—	—	—	—	—	—	—	32,500	—	—	—	454	—	—	—	11,500	46,454
Minn.	—	—	—	—	—	—	—	—	—	—	—	—	1,900	—	—	—	—	—	1,900
Totals	228,400	21,800	42,200	44,296	38,006	14,900	13,600	30,850	79,050	1,258,900	62	14,400	34,747	35,243	7,100	5,600	63,250	86,250	2,018,904

¹Maryland also received 18,000 *Ooencyrtus kuvanae.*

gregarious larvipositor that primarily prefers "hairless" lepidopterous larvae. It has been described as a minor parasite of the gypsy moth and the browntail, *Euproctis chrysorrhoea* (L.)= *Nygmia phaeorrhoea* (Donov), in Europe (Dowden 1933). The life history, behavior, and immature stages are very similar to the highly polyphagous *Compsilura concinnata* (Meigen). The hosts it will attack represent 15 families of Lepidoptera plus eight species of sawflies (Sabrosky and Reardon 1976). Although attempts to colonize this species from 1906 to 1932 failed in New England (Burgess and Crossman 1929, Dowden 1962), it is possible that much of the material released was *B. piniariae* (Hartig), rather than *B. nigripes* (Dowden 1933). Considering this, the parasite was reintroduced in 1974 from Austria and in 1975 from Poland.

Biology

B. nigripes has been in continuous laboratory production in Pennsylvania since November 1975. When reared in the laboratory at 24° C, it spends an average 10 days in the larval stage, 11 days in the pupal stage, and 14 days as an adult. Each prelarvipositing female contains an average of 140.8 eggs and larvae in its reproductive system. The parasite undergoes a prelarvipositing period of 5–7 days. Five days after adult emergence, the parasites are ready to larviposit. The favored laboratory host is late-instar *Galleria mellonella* (L.) (greater wax moth) larvae, averaging 50 percent parasitism. The parasite larva usually emerges from the host pupa. The parasite does not kill a significantly greater number of hosts than those that yield progeny. The usual sex ratio is 1:1.

Distribution

This species is found throughout the Palearctic region, Europe to Japan; introduced into New England but not established (Sabrosky and Reardon 1976).

Release and Recovery

See table 6.1–17. In 1976 and 1977, about 222,237 specimens of the fly were released in five different States.

Exorista japonica (Townsend)
William Metterhouse

Exorista japonica (Townsend) is a multivoltine, polyphagous, late-larva parasite of the "Japanese gypsy moth," *L. dispar japonicus* (Motschulsky). *Exorista japonica* material from Japan was first introduced in 1908, when it was poorly colonized, and again in 1910, when it was better colonized. Dowden (1962) recorded (as *Tachina*) that 471 individuals were released through 1927. This species did not become established (Sabrosky and Reardon 1976).

Biology

Probably overwinters as a maggot within overwintering host. Egg description and development compare closely to *E. segregata*. The maximum longevity of an individual female was 73 days; however, the average life is 15–20 days. The highest number of eggs layed by an individual was 379. The optimum period of fecundity was between 14 and 20 days. Within the laboratory the parasite is multivoltine.

Distribution

Parasite is native to Japan, China, and India; introduced into New England but not established.

Release and Recovery

See table 6.1–17. In 1977, NJDA released 642 adults in New Jersey. In 1978, NJDA and the Pennsylvania Bureau of Forestry released 89, 645, and 107,500 adults, respectively.

Exorista larvarum (Linnaeus)
William Metterhouse

Exorista larvarum (L.) is a multivoltine, late-larva parasite of the gypsy moth. This Palearctic species is exceedingly similar to the Nearctic *E. mella* (Walker), and probably misidentifications of both have been made (Sabrosky and Reardon 1976). *E. larvarum* is highly polyphagous. Herting (1960a) listed 54 species of nine families of Lepidoptera, including a number of important pests such as the gypsy, nun, satin, and browntail moths.

Biology

Overwinters as an immature maggot within an overwintering host. Biology similar to *E. segregata;* however, the maggot takes slightly longer to emerge from the egg (3–6 days). The maximum longevity of adults within the laboratory was 24 days. The highest number of eggs oviposited by an individual female was 124. The parasite was multivoltine within the laboratory.

Distribution

Parasite is widespread in the Palearctic region, Europe, and North Africa to Japan; introduced and established into Northeastern United States.

Release and Recovery

See table 6.1–17. *E. larvarum* has been recovered at New Jersey release sites by the NJDA only during the same year of releases. This species was first released in New Jersey 1970; the first record of recovery is in 1974 in Burlington and Middlesex Counties. Because of the taxonomic difficulties in distinguishing *E. larvarum* from the native species *E. mella,* it is doubtful that *E. larvarum* has actually been recovered in New Jersey; the recoveries were probably *E. mella.* Three specimens of *E. larvarum* were recovered from gypsy moth during 1974 in Pennsylvania. These specimens were collected from a site where *E. larvarum* had been released that same season. *L. dispar* larvae have been collected and reared for emergence of *E. larvarum* since 1973, but no other specimens have been recovered. Reardon (1976) recovered six specimens of *E. larvarum* (two each in Cobleskill, N.Y., and Cape Cod, Mass., and one each in Whitehall, N.Y., and Clinton, N.J.) from host collections made within the Forest Service Intensive Plot System.

Exorista rossica Mesnil
William Metterhouse

Exorista rossica Mesnil is a multivoltine, late-larva parasite of the Indian gypsy moth, *L. obfuscata* (Walker). In the United States, this species was readily colonized in the laboratory on *L. dispar.* It was recorded from *Leucoma salicis* (L.) in Armenia by Richter (1968). Abdullaev (1966) recorded it as a

parasite of *L. dispar* in Uzbekistan and also noted that in the Caucasus it was an effective parasite of *S. salicis* and *Malacosoma neustria.*

Biology

Overwinters in host larvae or pupae. Egg description and maggot development are similar to *E. segregata.* Females were recorded as laying 250 eggs over 25 days. Adult longevity of 35 days was noted in the laboratory. Rao (1964) credits *E. rossica* with completing three generations on *L. obfuscata* in Kashmir. Adults of the first generation appear in the field by the beginning of May. The second generation starts early in June and the third generation toward the beginning of July. Eggs hatch in 2 to 3 days depending on the temperature. Larval development requires from 13 to 16 days. The mature larva, which is milky white, pupates in approximately 8 hours. The pupal period requires 12 to 13 and 13 to 14 days for males and females, respectively. Egg laying continues for about 25 days, during which about 250 eggs are laid. In one case a female laid 38 eggs in one day.

Distribution

Parasite native to Armenia, Uzbekistan and Tadzhikiatan (U.S.S.R.), and northern India; introduced into United States but not established.

Release and Recovery

See table 6.1–17. There is no record of this species ever being recovered in any of the areas where it was released.

Exorista segregata (Rondani)
William Metterhouse

Exorista segregata (Rondani) is a multivoltine, polyphagous, late-larva parasite of *L. dispar.* This species was apparently introduced early in the program on biological control of the gypsy moth, but it was confused with *E. larvarum,* so that the exact numbers in 1906 and 1907 are not known. A large colony was released in 1909 and additional individuals in 1911, but the species did not become established. Specimens were doubtfully recovered in 1909. A few

Table 6.1-17.—*Species of gypsy moth parasites released for establishment or redistribution, 1963–77*

Parasite species	Released by	Release site	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	Total
Tachinidae																		
Blondelia nigripes	Pa. Bur. For.	Pa.														1,300	114,958	116,258
		N.J.														22,800	74,640	97,440
		Del.														1,600	5,200	6,800
		N.Y.														250		250
		W.Va.														1,489		1,489
Exorista japonicus	N.J. Dep. Agric.	N.J.															642	642
Exorista larvarum	N.J. Dep. Agric.	N.J.								3,042	3,780	46,360	73,870				14,900	141,952
	N.C. Dep. Agric.	N.C.														1,350		1,350
	Pa. Bur. For.	Pa.										250	492	64,279	108,038			173,059
Exorista rossica	N.J. Dep. Agric.	N.J.							11,794	59,780	9,632	32,976						114,182
	USDA (Otis lab)	Mass.					90	690	2,195	2,926	7,750							13,651
		Conn.						1,342			4,025							5,367
		N.H.									3,850							3,850
		Pa.							1,973		3,600							5,573
Exorista segregata	N.J. Dep. Agric.	N.J.		5,780				5,465	30,289	68,478	9,875	33,464						153,351
	Va. Dep. Agric. & Commerce	Va.													200	159		359
	USDA (Otis lab)	Conn.	7,361					4,651										12,012
		Mass.			63		1,166	1,378	3,122	2,116								7,845
		Vt.		2,400														2,400
		N.Y.		2,200														2,200
	Pa. Bur. For.	Pa.							5,762			748			25,000	48,864	10,407	90,781
	Md. Dep. Agric.	Md.											2,818					2,818
Palexorista disparis	N.J. Dep. Agric.	N.J.										17,887	32,879	112,950				163,716
	Va. Dep. Agric.	Va.												800	600			1,400
	USDA (Otis lab)	Mass.									50							50
Palexorista inconspicua	N.J. Dep. Agric.	N.J.												22,300	31,684	175,266		229,250
	USDA	Mass.									1,075							1,075
		N.H.									250							250
		Conn.									60							60
	Conn. Agric. Exp. Stn.	Conn.												800				800
	Pa. Bur. For.	Pa.										151	275	222,847	344,141			567,414
Ichneumonidae																		
Coccygomimus disparis	N.J. Dep. Agric.	N.J.										600	73,795	43,413	48,982	4,619	33,980	205,389
	W.Va. Dep. Agric.	W.Va.												2,200	1,760			3,960
	Pa. Bur. For.	Pa.												419			1,833	2,252
Coccygomimus instigator	N.J. Dep. Agric.	N.J.											46,492		49,420			95,912

Table 6.1-17 (cont.)

Parasite	Agency	State	Release figures (by column, left→right)	Total
Coccygomimus turionellae moraguesi	N.J. Dep. Agric.	N.J.	63,050; 39,000	102,050
Coccygomimus turionellae turionellae	Md. Dep. Agric.	Md.	9,453; 8,874	18,327
	N.J. Dep. Agric.	N.J.	2,200; 122,558; 217,206; 104,050; 3,430	449,444
	Va. Dep. Agric.	Va.	1,800; 16,125; 10,250; 10,716; 453	39,344
	N.C. Dep. Agric.	N.C.	5,150; 11,200	16,350
	Pa. Bur. For.	Pa.	4,531; 12,579	17,110
Braconidae				
Apanteles liparidis	Pa. Bur. For.	Pa.	4,500; 403,333; 817,173; 4,000; 121,820	1,350,826
		Del.	3,000; 19,100; 28,500; 17,750	68,350
		N.C.	1,900	1,900
		N.Y.	4,500; 1,000; 250	5,750
		W.Va.	2,141; 3,241; 3,168	8,550
		N.J.	3,985; 25,850; 381,250; 643,800; 286,810; 214,625	1,556,320
Apanteles porthetriae	Pa. Bur. For.	Del.	1,000; 1,250	2,250
		N.C.	990	990
		Mass.	2,862; 217; 24,686	27,503
		Conn.	36; 4,017	6,879
		N.J.	137; 178; 16,000; 26,615; 64,120	107,086
Meteorus pulchricornis	Pa. Bur. For.	Pa.	2,415; 8,188; 115,531; 8,050; 14,015	148,199
		Del.	2,500; 500; 1,200; 6,001	10,201
		Wis.	250	250
		N.C.	2,020; 2,480	2,500
		N.Y.	750; 700; 275	1,725
		W.Va.	205; 610; 461	1,276
		N.J.	3,416; 41,886; 23,247; 17,358; 30,262	116,169
Rogas indiscretus	N.J. Dep. Agric.	N.J.	295; 1,127; 1,796; 2,523; 8,133; 10,227	24,101
	USDA (Otis lab)	Mass.	525; 205; 3,339	4,069
		Conn.	1,455	1,455
		Pa.	1,217	1,217
Mermithidae				
Hexamermis albicans	N.J. Dep. Agric.	N.J.	17	17
	Pa. Bur. For.	Pa.	17	17
Chalcididae				
Brachymeria intermedia	N.J.	N.J.	300; 4,955; 1,846; 138,705; 69,345; 258,253; 373,529; 368,295; 254,650; 14,527; 36,598; 43,800; 65,500	1,630,303
	Univ. of Maine (Leonard)	Maine	10,000; 11,000; 14,000	35,000
	N.C. Dep. Agric.	N.C.	2,495; 1,340; 2,800	6,635
	USDA (Otis lab)	N.Y.	1,550	1,550
	Syracuse Univ.	N.Y.	77,000	77,000
	USDA	Conn.	10,636	10,636
	Va. Dep. Agric. & Commerce	Va.	1,000; 21,000; 8,250; 6,000; 1,250	37,500
	W.Va. Dep. Agric.	W.Va.	23,080; 43,980; 135,430; 11,290	213,780
	Pa. Bur. For.	Pa.	5,572; 3,767; 6,859; 9,500; 14,076; 189,665; 66,883; 148,104; 3,789	448,215
	Md. Dep. Agric.	Md.	19,500; 23,632; 2,000; 33,638	78,770

hundred specimens were colonized in 1924–27, from Spain, Portugal, and Algeria, but again they did not become established. However, the species was known to be an excellent parasite of *L. dispar* in southern Europe, especially in Spain, and introductions into New England were resumed in the late 1940's. In 1967, puparia were brought from Spain to the USDA laboratory at Moorestown, N.J., and then transferred to the USDA laboratory at Otis Air Force Base, Mass., for propagation and release of adults.

Biology

Overwinters as an immature maggot within an overwintering host (Sabrosky and Reardon 1976). Female deposits a large, white, macrotype egg. Eggs are deposited on the integument of the host with a preference for the pleural anterior section. The maggot emerges from the egg within 2–5 days and immediately enters the host. A bruise mark will appear around the entrance wound within 48–72 hours. Egg viability varies with 50–75 percent emergence from eggs layed by females 3–4 days old, and 75–100 percent emergence from females 5 days old or older. In the laboratory, maggots pupate within or outside of host larvae or pupae in 8–12 days. Adult flies emerge 9–13 days after pupating with males emerging before females. Virgin females are not parthenogenic and lay nonfertile eggs. Mating usually occurs within 24 hours after emergence and lasts from a few minutes to several hours. Mating sometimes occurs more than once. Females have been recorded laying 531 eggs over 33 days. Optimum egg production occurs at 6–15 days. Adult longevity averages 12–18 days.

Distribution

Parasite is native to southern Europe and northern Africa; introduced into Northeastern United States but not established.

Release and Recovery

See table 6.1–17. One specimen of *E. segregata* was recovered in Pennsylvania during 1977 from a larva of *Malacosoma americanum* (F.). *E. segregata* was released at this recovery site during 1977. *E. segregata* is not yet known to be established in North America.

Palexorista disparis Sabrosky
William Metterhouse

Palexorista disparis Sabrosky is a multivoltine, late-larva parasite of the "Indian gypsy moth," *L. obfuscata*. Rao (1967) recorded this species as "*Drino discreta*," one of the principal parasites of *L. obfuscata* in northern India. The Loshta specimens appear to be part of those recorded by Rao (1967) as *sinensis*, a "very prominent parasite of *L. obfuscata*." As *discreta*, it was colonized on *L. dispar* at Otis Air Force Base, Mass., and released in New England on a limited scale. Later it was propagated and released in New Jersey and then in Pennsylvania (Sabrosky and Reardon 1976).

Biology

Probably overwinters within the larvae of the host. Egg description and development are similar to *P. inconspicua*. Females will lay 90–115 eggs. Males usually succumb within 10–15 days after emergence; females live for 20–25 days within the laboratory. Under laboratory conditions the parasite is multivoltine.

Distribution

This species is found in northern India.

Release and Recovery

See table 6.1–17. *P. disparis* has not been recovered in North America.

Palexorista inconspicua (Meigen)
William Metterhouse

As *Sturmia inconspicua*, from central Europe, the species was reared at the Gypsy Moth Laboratory in Massachusetts from 1906 to 1911 and then released during some of those years. This effort was repeated in 1923–28. The species did not become established from the first series of releases, but from the second series at least one colony in Massachusetts was established for a few years (first recovery, 1929); apparently,

however, it was not established permanently (Clausen 1956). Herting considered *inconspicua* as primarily a parasite of diprionid sawflies, although it was also common on various lepidopterous larvae. Webber (1932), while admitting that it was not as good a parasite of *L. dispar* as *Parasetigena* or *Blepharipa*, believed that it "compares favorably" with other tachinids, and in some localities it was even better than *Blepharipa* "*scutellata*" (*pratensis* (Meigen)). As either *Sturmia* or *Drino inconspicua*, the species has been recorded several times in northern India. The Indian form was first imported at Otis Air Force Base, Mass., and then further propagated and released in New Jersey, beginning in 1971, before the importation of *inconspicua* from southern France.

Biology

Overwinters as a first-instar larva in cocoons of diprionid sawflies and in overwintering larvae of a pine caterpillar, *Dendrolimus pini* (L.) (Sabrosky and Reardon 1976). A transparent macrotype egg is usually oviposited on the pleural or the ventral surface of the gypsy moth larva. The embryo at the time of oviposition is fully matured and enters the host within 15 minutes to 1 hour. The highest number of progeny recorded from one female was 122, the lowest 82. Nonfertile females will not oviposit. The maximum longevity of a female adult is 60 days. Maggots usually emerge from host larvae 5–9 days after oviposition. Very few maggots have been observed emerging from host pupae. Adult parasites emerge 10–14 days after pupation, males emerging a day or two before females. Female parasites will mate within 24 hours and often more than once. There is a preoviposition period of 5–7 days. The parasite is multivoltine and superparasitism within the laboratory is common.

Distribution

This species is found in Continental Europe, North Africa, and North India (Kashmir, Himachal Pradesh, Uttar Pradesh).

Release and Recovery

See table 6.1–17. One specimen of *P. inconspicua* was recovered from *L. dispar* in 1974 in Pennsylvania

(Fusco 1978). The parasite was released in the recovery site during 1974, but *P. inconspicua* is not known to be established in Pennsylvania.

Palexorista solennis (Walker)
William Metterhouse

Palexorista solennis (Walker) is a multivoltine, late-larva parasite of the "Indian gypsy moth," *L. obfuscata*. As far as known, this species was not introduced, although as *sinensis* (part) it was recorded by Rao (1967) as "a very prominent parasite of *L. obfuscata* in Himachal Pradesh," with parasitism ranging from 12–27 percent (Sabrosky and Reardon 1976).

Distribution

This species is widely distributed in the Oriental region and the islands of the Southwest Pacific: India and Ceylon to China (Shanghei), Taiwan, and the Mariannas to Tongo, North Queensland, and Indonesia (Sabrosky and Reardon 1976).

Biology

Not described.

Release and Recovery

There is no record of this species ever being released or recovered in North America.

Ichneumonidae

Coccygomimus disparis (Viereck)
William Metterhouse

Coccygomimus disparis (Viereck) (or sp.) is a polyphagous pupal parasite of the gypsy moth.

Biology

Information on overwintering is not available, but its biology is similar to *C. turionellae turionellae*. The highest number of progeny per female recovered from parasitized gypsy moth prepupae was 199. The parasite within the laboratory is multivoltine and solitary.

Distribution

This species is widely distributed in China, Izu-shichito Islands, India (?), Japan, Korea, and Sakhalin (Townes et al. 1965).

Release and Recovery

See table 6.1–17. Ten specimens of *C. disparis* were recovered in New Jersey during 1978. *C. disparis* is not yet known to be established in North America.

Coccygomimus instigator (Fabricius)
William Metterhouse

Coccygomimus instigator (F.) is a polyphagous pupal parasite of the gypsy moth.

Biology

Similar to *C. turionellae turionellae*, within the laboratory this species is multivoltine and solitary. Information on overwintering is not available.

Distribution

This species is widely distributed in China, Germany, Japan, Korea, Kuriles, Russia, and Sakhalin (Townes et al. 1965).

Release and Recovery

See table 6.1–17. This species has not been recovered in North America.

Coccygomimus turionellae moraguesi (Schmiedeknecht)
William Metterhouse

Coccygomimus turionellae moraguesi is a polyphagous pupal parasite of the gypsy moth.

Biology

Information on overwintering is not available. Biology is similar to *C. turionellae turionellae*. The highest number of progeny per female recovered from parasitized gypsy moth prepupae was 188. The parasite within the laboratory is multivoltine and solitary.

Distribution

This subspecies is widely distributed in Austria, Japan, Kamchatka, Korea, Kuriles, North Africa (?), North America, Russia, Ryukyu Island (?), Sakhalin, and Sweden (Townes et al. 1965).

Release and Recovery

See table 6.1–17. This subspecies has not been recovered in the release States.

Coccygomimus turionellae turionellae (Linnaeus)
William Metterhouse

Coccygomimus turionellae turionellae (L.) is a polyphagous pupal parasite of the gypsy moth.

Biology

No information is available on overwintering biology. The parasite oviposits an egg within host prepupae or pupae. All developmental stages are completed within host. Adult parasites emerge from host 15–30 days after oviposition. Mating occurs within 24 hours after emergence, with multiple mating being observed. A preoviposition period of 5–9 days is usually required. The average number of progeny per female recovered from parasitized gypsy moth prepupae was 81, the highest 134. The sex ratio is usually 2:1 in favor of males. Females are parthenogenic and, if unmated, produce males. The longevity of females is 24–30 days, with mortality occurring earlier in males. Within the laboratory, the parasite is multivoltine and solitary. The parasite prefers to oviposit in prepupae rather than pupae. As pupae chitinize with maturity, ovipositing becomes difficult. Ovipositing in pupae occurs through intersegmental folds. Parasitized prepupae usually do not complete pupation.

Distribution

The species is widely distributed in Austria, Japan, Kamchatka, Korea, Kuriles, North Africa (?), North America, Russia, Ryukyru Island (?), Sakhalin, and Sweden (Townes et al. 1965).

Release and Recovery

See table 6.1–17. This subspecies has not been recovered in the release states.

Hyposoter tricoloripes (Viereck)
Robert A. Fusco

Hyposoter tricoloripes (Viereck) is a major parasite of the gypsy moth in some areas of Europe. It is a multivoltine, solitary ichneumonid that attacks small gypsy moth larvae. Unlike its close relative, *Phobocampe disparis* (Viereck), those individuals which attack gypsy moth do not diapause. Therefore, this species apparently requires an alternate host(s). In Europe, it appears to be most effective at low gypsy moth population densities, but its distribution is spotty and effective only in certain locations. *H. tricoloripes* was introduced to the United States from France in 1972, from Germany and Austria in 1974, and from Germany, Austria, and Poland in 1975.

Biology

Attempts were made to laboratory rear *H. tricoloripes* in Pennsylvania on two occasions: June–October 1975, and June–July 1976. When reared in the laboratory at 24° C, it took 12–16 days from oviposition until parasite larvae emerged and formed cocoons. Adult parasites emerged 7–14 days later. Females mated immediately upon emergence from the cocoons. The favored laboratory host was first-instar *L. dispar* larvae, but it oviposited also in second- and third-instar larvae. The most successful rearing attempt occurred with a 30×30×30 cm screen-covered oviposition cage supplied with honey smears and distilled water. Initially, the cage contained 36 male and 36 female parasites. Fifty to 150 first-instar *L. dispar* larvae were placed into the cage for 24 hours. The larvae were removed and placed on synthetic gypsy moth diet in 473-ml containers until the parasites emerged and formed cocoons. All rearing was done at 24° C+1.5 about 30 percent RH and with a 14-hour photophase. An extremely poor rate of parasitism (<5 percent) and an equally poor sex ratio (nine males to one female) were obtained. Smaller oviposition containers were tried with even less success.

Release and Recovery

It was never mass cultured successfully by either the NJDA or the Pennsylvania Bureau of Forestry laboratories. Therefore, no colonization attempts were made. Nevertheless, twenty-five (12 females, 13 males) *H. tricoloripes* were released in a field cage containing 1,000 first-instar gypsy moth larvae to study the parasite's behavior. The cages have been described previously (Pennsylvania Department of Environmental Resources 1976). Female parasites were more aggressive under field-cage conditions than in the laboratory, actively parasitizing moving gypsy moth larvae after a rapid searching pattern over the leaves. Searching was restricted to shaded areas under the canopy. Adult parasites were observed in the cage for up to 8 weeks, but no parasite cocoons were found until the fall when three empty *H. tricoloripes* cocoons were found on leaves in the leaf litter. All three of the cocoons had emergence holes from hyperparasites.

Braconidae

Apanteles lacteicolor Viereck
Robert A. Fusco

Apanteles lacteicolor Viereck, primarily a parasite of the browntail moth, became established in the United States in 1908 (Dowden 1962). The parasite was first described by Viereck (1911) from material reared at the Gypsy Moth Laboratory, Melrose Highlands, Mass. Its life history and biology were discussed by Musebeck (1918). The early colonization efforts were described by Burgess and Crossman (1929).

Biology

The parasite is multivoltine and solitary and attacks early-stage larvae. It overwinters as a first-instar larva in the browntail moth and occasionally attacks small gypsy moth larvae in May and June. It is found only where browntail moth infestations occur.

Release and Recovery

Recent importations of the parasite were made from France in 1975 and from Iran and India in 1976 and 1977. No field releases have been made recently.

Apanteles liparidis (Bouché)
Robert A. Fusco

More effort has been expended in recent years in attempting to colonize Apanteles liparidis (Bouché) than any other exotic parasite. It is probably the most important parasite of the Japanese gypsy moth, L. dispar japonica and is a major parasite of L. dispar in some areas of Europe. The early colonization efforts, description, life history, and biology were described by Burgess and Crossman (1929) and Wilkinson (1945). The parasite is oligophagous and gregarious and has two generations on the gypsy moth. It requires an overwintering host(s) that apparently is not present in the United States. In Europe it overwinters as an immature larvae in Dendrolimus pini (L.). In Japan it has been reported to overwinter in D. spectabilis (Burgess and Crossman 1929).

Biology

A European strain of A. liparidis was reared in Pennsylvania from February 1974 until January 1977. A Japanese strain has been reared from July 1976 to the present. When reared at 24° C it takes an average 18 days from oviposition until the parasite larvae emerge and form cocoons. The adults emerge from the cocoons in an average 5.5 days. Adults will live 7–14 days depending on oviposition rate. In the laboratory, the parasite prefers third-instar L. dispar larvae but has been reared on fourth- and fifth-instar larvae as well. In addition, the Japanese strain of A. liparidis has been reported to prefer second-instar gypsy moth larvae in the field. Efforts to rear that strain in the lab on second-instar larvae were not as successful as with third-instar larvae. Each female contains an average 166 eggs in its reproductive system. An average of 8.5 parasites is obtained per host exposed to the European strain, and approximately 70 percent of the hosts exposed to that strain are parasitized. An average of two parasites is

obtained per host exposed to the Japanese strain in the laboratory; less than 50 percent of the hosts exposed to this strain are parasitized. In both strains, the sex ratio varies greatly from generation to generation, but overall, an average 1:1 ratio is obtained. Fukaya (1938) discusses the effect of temperature and humidity upon the development of A. liparidis.

Release and Recovery

During the early attempts at colonization of A. liparidis in New England, 116,952 individuals were released (Dowden 1962). The majority of these individuals were released in Massachusetts. In recent years, 2,987,696 specimens were released in 60 counties and seven States (table 6.1–17). Although the majority of these parasites were released into gypsy moth infestations, many of the releases were directed at "potential" alternate hosts in states not having the gypsy moth or at a time when gypsy moth larvae were no longer available for parasitization. In a field-cage study, A. liparidis completed two generations on the gypsy moth during the host feeding period. The parasite readily attacked first- and second-instar larvae on foliage (usually in early May), and their progeny emerged in time to attack late-stage hosts. The second generation emerged without any incidence of diapause and searched for another host to sustain itself or in which to overwinter. Gypsy moth larvae provided at this time will be attacked, but the parasites will not enter diapause.

In a similar study, attempts were made to induce diapause by exposure to lower temperatures and photophase. In September 1976, 200 fourth-instar gypsy moths were introduced into a cage which had continually sustained A. liparidis throughout the summer. After the parasite had completed two normal generations on the gypsy moth, two more generations were completed on seeded gypsy moth larvae. The parasites readily attacked these late-season larvae, inducing 68 percent parasitism. Adult A. liparidis began emerging in late October and continued to emerge until lower temperatures killed these slower developing parasites. Many of the parasite cocoons contained the hyperparasites Gelis

obscurus (Cresson) and *Gelis apantelis* Cushman (determined by R. W. Carlson). Diapause induction was not accomplished.

On the basis of the above tests, it is apparent that *A. liparidis* in North America is able to complete two generations on gypsy moth but must have an alternate overwintering host. Another section of the compendium presents information on attempts to find suitable alternate hosts.

Apanteles ocneriae Ivanov
Robert A. Fusco

Apanteles ocneriae Ivanov is a rare braconid parasite reported only from the U.S.S.R. and Yugoslavia (Telenga 1955, Hackett 1971, Vasić 1976). It has been described to be closely related to *A. solitarius* (Ratz.) (= melanoscelus (Ratz.)), differing in the coloration of the antennae, palpi, legs, and cocoons.

Biology

The gypsy moth is apparently its only host. *A. ocneriae* cocoons are white, connected by filaments but not enclosed in a common web (Telenga 1955).

Release and Recovery

Although it has never been purposely introduced into the United States, apparently it was present in a shipment of parasites labeled *Apanteles* spp. shipped to the United States from Yugoslavia (Chianese 1978). It was laboratory reared for a short period by the NJDA in 1970 as a mixed colony of *Apanteles* but was never released in the United States.

Apanteles porthetriae Muesebeck
Robert A. Fusco

Apanteles porthetriae Muesebeck, a multivoltine, oligophagous, solitary parasite, attacks small larvae of the gypsy moth. It was imported from Europe in 1922 (Muesebeck 1928) where it was described as being a major parasite of the gypsy moth (Burgess and Crossman 1929). It also has been reared from *Lymantria obfuscata* in India (Rao 1966). It has been

reared and released rather extensively in the United States both during the early years of the gypsy moth biological control efforts and recently. It has not become established. It has only one generation on the gypsy moth and must find alternate hosts to sustain itself and in which to overwinter. Additional details on various alternate hosts studies are discussed in another section of the compendium.

Biology

Apanteles porthetriae has been in continuous production in Pennsylvania since April 1974. When reared in the laboratory at 24° C, 16 days are required between oviposition and cocoon formation. Adults emerge in an additional 6 days and mate shortly thereafter. Adult females will live for 7-21 days depending on the rate of oviposition. This parasite prefers late first-instar or early second-instar *L. dispar* larvae. Each female contains an average 68.7 eggs in its reproductive system. Parasitism averages 60 percent with only one parasite being produced per host parasitized. In the laboratory, exposed hosts are maintained at 24° C±1.5, about 30 percent RH and a 14-hour photophase, until the parasite larvae emerge and form cocoons. An overall sex ratio of 1:1 is obtained with fluctuation occurring from generation to generation.

Release and Recovery

Burgess and Crossman (1929) listed 27,887 adult *A. porthetriae* and 6,700 parasitized larvae as having been liberated in 10 Massachusetts and two New Hampshire towns from 1924 to 1927. Recently, during 1964-1977, 349,984 parasites were released in 53 counties and eight States (table 6.1-17).

Apanteles ?ruidus Wilkinson
Robert A. Fusco

Apanteles ruidus Wilkinson is a solitary, multivoltine, early-larva parasite of the "Indian gypsy moth," *L. obfuscata*. Though originally determined as *"ruidus"*, it is probably not that species. Taxonomic study of the species is continuing. It was introduced from India in 1977 and is currently being reared in

several laboratories on the North American gypsy moth. This parasite species is closely related to *A. melanoscelus* (Wilkinson 1928) and, if colonization is successful, may be a valuable addition to the North American fauna. Pimentel (1963) discusses the value of introducing parasites of closely related host species in biological control work. *A. ruidus* readily attacks *L. dispar* in the laboratory but to date has not displayed any incidence of diapause in this host. Its alternate host requirements are not known.

Biology

Apanteles ?ruidus has been in production in Pennsylvania since November 1977. When reared in the laboratory at 24° C, it takes 13 days between oviposition and cocoon formation. An average of 6.5 days is required between cocoon formation and adult emergence. Adults live for 7–21 days. The parasite prefers late first-instar or early second-instar *L. dispar* larvae. Each female contains an average 85.4 eggs in its reproductive system. Parasitism has averaged about 70 percent with only one parasite being produced per host parasitized. The sex ratio has been two males to one female.

Release and Recovery

No releases have been made to date in the United States.

Meteorus pulchricornis (Wesmael)
Robert A. Fusco

Meteorus pulchricornis (Wesmael) is a solitary, multivoltine, polyphagous wasp that prefers second- and third-instar *L. dispar*. It is not considered an important parasite of *L. dispar* in Europe (Burgess and Crossman 1929).

Biology

Meteorus pulchricornis was reared in Pennsylvania from April 1974 until November 1977. When reared in the laboratory at 24° C, it spends an average of 3 days in the egg stage, 9.5 days in the larval stage, 8 days in the pupal stage, and 35 days as an adult. Each

preoviposition female contains an average of 49.5 eggs in its reproductive system. The preferred laboratory host is third-instar *L. dispar* larvae with an average rate of parasitism of 70 percent. The normal sex ratio of the progeny is 1.25 females to one male.

Release and Recovery

One hundred twenty-two adults of *M. pulchricornis* were released in New England in 1922. These were the progeny of two male and two female specimens from southern France. Importation and release resumed in 1972. Approximately 282,320 adults were released in 67 counties in seven States from 1972 to 1977 (table 6.1–17). In 1975, a single recovery of a *Meteorus* species, tentatively identified as *pulchricornis*, was made from a Malaise trap in Wisconsin. This was recovered 2 years after the release of 250 parasites near Black River Falls, Jackson County (Hall 1978). In a field-cage study conducted in 1976, *M. pulchricornis* displayed an excellent searching ability, was fairly aggressive and a strong flier, and had a high innate capacity for increase. Parasitized gypsy moth larvae and parasite cocoons were found on understory shrubbery or on the lower bole of host trees. This would indicate that sampling for establishemnt purposes should be directed in these locations.

Rogas indiscretus Reardon
William Metterhouse

Rogas indiscretus Reardon is a solitary endoparasite of second- and early third-instar larvae of *L. dispar*.

Biology

An egg is deposited inside the body of the host. Oviposition lasts 0.5 to 3 minutes. A female can lay 10 to 15 eggs per day and at least 143 eggs in her lifetime. Pupation of the immature parasite occurs 16 to 30 days after oviposition. The mature maggot pushes the unconsumed body contents out of the larval skin on the anteroventral surface and spins a cocoon within the host integument (Clausen 1972). The resulting mummy is shortened, dorsally convex, and firmly

attached to the substrate by the exuded host material. Emergence of the adult parasite occurs from the dorsal side on the posterior end of the mummy 16 to 19 days after pupation, males emerging 2 or 3 days in advance of females. Adults live from 21 to 38 days and begin parasitizing after a preovipositional period of 4 to 5 days. Developmental times within the life cycle show great variation both between seasons and between individuals. In the fall, the incidence of diapausing mummies increases sharply. Reardon (1973) encountered 80 percent diapause in mummies formed in September and October. The length of diapause and the percent recovery of adult parasites from dormant mummies are also highly variable.

In the laboratory, *R. indiscretus* is an inactive parasite difficult to induce to mate or to oviposit. Reardon (1973) recovered mummies from 59 percent to 68 percent of the larvae stung, while the NJDA in 5 years of rearing averaged 20 percent parasitism. Inadequate mating has been a major obstacle to mass rearing. Although a flurry of activity and increased mating behavior frequently follow a rapid shift in temperature, humidity, or light intensity or quality, the conditions that lead to successful mating on one occasion often have no effect on another. Mated females produce male and female progeny while unmated females are parthenogenic, producing male progeny. Sex ratios of successive generations are variable with males usually in the majority.

Distribution

Parasite native to India; introduced into the United States but not established (Marsh 1978).

Release and Recovery

See table 6.1-17. A total of 30,842 adults was released in New Jersey, Massachusetts, Connecticut, and Pennsylvania.

Rogas lymantriae Watanabe
William Metterhouse

Rogas lymantriae Watanabe is a solitary larval endoparasite of *Lymantria dispar* on the island of Honshu, Japan.

Biology

The parasite develops from the egg to pupal stage in 13–14 days. Pupation occurs in a mummy similar to that described for *R. indiscretus* and from which adults emerge in 8–10 days. Diapause may occur while the parasite is a mature maggot (prepupa?) within the mummy. High humidity appears necessary for increased survival of diapausing parasites. *R. lymantriae* attacks late third-instar of *L. dispar*. No alternate host has been encountered. Like *R. indiscretus*, *R. lymantriae* is difficult to mate in captivity. No set of conditions has been discovered that will reliably induce mating. Sex ratios of lab-reared progeny have ranged from 1 to 2.3 females to one male.

Distribution

The parasite is found in Japan; it was introduced into the United States but is not yet field released or established (Marsh 1978).

Release and Recovery

This species has not yet been released in the United States, although it is being reared for future release.

Chalcididae

Brachymeria "euploeae" (Westwood)
Robert A. Fusco

Brachymeria "euploeae" (Westwood), imported from India in 1977 from *L. obfuscata*, may be the *B. hearseyi* var. *xanthoterus* Waterston of Joseph et al. (1973). Though originally determined as *"euploeae"* it is probably not that species. Taxonomic study of this species is continuing. In earlier literature it was referred to as *Chalcis. B."euploeae"* is a pupal parasite that reproduces uniparentally by deuterotoky. Deuerotkous species usually are obligatorily parthenogenic but occasionally will produce a few males. These males have no function in reproduction.

Biology

Brachymeria "euploeae" has been reared in Pennsylvania since January 1978. When reared at 25° C, it

takes approximately 21 days from oviposition until adult emergence (parasite pupates within host). The parasite may be reared on either *L. dispar* or *G. mellonella* pupae. The rate of parasitism has been approximately 30 percent. The rearing is the same as for *B. lasus.*

Release and Recovery

Release of this species is being held up pending results of biological studies relating to potential facultative hyperparasitic tendencies.

Brachymeria lasus (Walker)
Robert A. Fusco

Brachymeria lasus (Walker), also referred to as *Chalcis* and *B. obscurata* (Walker) in earlier literature, is a polyphagous pupal parasite, very similar in behavior to *B. intermedia* (Nees) (Burgess and Crossman 1929). It is widely distributed throughout southeastern Asia, including Japan, India, and the Philippines and in Hawaii (Joseph et al. 1973). All of the importations into North America have been from Japan. Although under laboratory conditions it will attack and reproduce in various tachinid puparia, it is believed to be a primary parasite of only lepidopterous pupae. In contrast to *B. intermedia*, which is found primarily in dense gypsy moth populations, there is speculation that *B. lasus* may be a low host density gypsy moth parasite, as it is collected from sparse populations of *L. dispar japonica. B. lasus* is a minor parasite in Japan. It apparently overwinters in the milder climates of Japan as an adult. There is some question of its ability to overwinter in the colder climate of North America. The alternate host requirements are not known.

Biology

Brachymeria lasus has been reared in Pennsylvania since July 1977. When reared at 25° C, it takes an average 21 days from oviposition until adult emergence (parasite pupates within host). Ovipositing adult females live for 3–6 weeks. After a 48-hour mating period at 25° C, adults are either removed for ovipositing or stored at 13° C±1.5 and 8-hour

photophase followed by 7° C±1.5, 7-hour scotophase and 9-hour subdued (twilight) photophase. The storage temperature is raised to 24° C for 6 hours a week to allow the parasites to feed. The parasite has been reared equally well on *L. dispar* and *Galleria mellonella* pupae. The rate of parasitism has been approximately 25 percent. An average 1:1 sex ratio has been obtained.

Release and Recovery

Dowden (1962) reports the release of 394 *B. obscurata* in North America in 1908 and 1909. No recoveries were made. Release of this species was held up until studies of potential facultative hyperparasitic tendencies were completed.

Nematoda: Mermithidae
Jack R. Coulson

In addition to the dipterous and hymenopterous parasites discussed above, nematode parasites have been recovered from gypsy moth larvae during foreign explorations and released against *L. dispar* in the United States in recent years. Although sometimes classed as "pathogens," entomophagous nematodes attack and interact with their insect hosts in much the same manner as the insect parasites discussed above, and are thus discussed as parasites in this book.

Nematodes collected from gypsy moth larvae in both Europe and Japan have all been identified by W. R. Nickle, Nematology Laboratory, Beltsville, Md., as *Hexamermis albicans* (Siebold) (Nematoda: Mermithidae). According to Nickle (1978*a*), this "species," which has a long host list, primarily Lepidoptera (Stiles and Hassall 1920, Nickle and Grijpma 1974), may in fact represent a species-complex. Thus, several species may be involved in the collections made from gypsy moth larvae in Europe and Japan.

Biology and Importations

There is also little currently known about the biology of these nematodes. The nematodes have issued as juveniles or immatures from late-stage gypsy

moth larvae, originally collected in first, second, or third instars (Drea et al. 1977). These juveniles then apparently enter the soil to overwinter, transform to the adult stage, mate, and lay eggs (Nickle 1978*a*). How the new generation of nematodes effect parasitism of host larvae is not yet known.

Hexamermis "albicans" was first reported as a parasite of the gypsy moth in the Voronezh Region of the U.S.S.R., where 60 percent mortality of gypsy moth larvae was attributed to its parasitism (Artyukhovskii 1953). Because of this report, *H. "albicans"* was among the material requested from the U.S.S.R. by ARS as part of the exchange of biological control agents between the United States and the U.S.S.R. As a result, 30 immature nematodes were received from the U.S.S.R. in early 1976 and forwarded from the ARS quarantine facility to the ARS Nematology Laboratory of Beltsville. Most of these were dead, but a few molted to adults in the laboratory, although they failed to reproduce. These preserved specimens are being held for taxonomic study (Nickle 1978*a*).

The nematode was first encountered by ARS explorers in 1974 during work in Austria and Germany and again in 1975 in Austria (Drea et al. 1977). The species was recovered from both the gypsy moth and the satin moth, *Leucoma salicis* (L.). A total of 41 juvenile nematodes was sent to the United States in 1974, primarily from Austrian collections, only three being from Germany. These were forwarded by the quarantine station to Beltsville. Of these, 17 were released (placed in the soil under trees infested with gypsy moth egg masses) on July 16, 1974, near Barbertown, Hunterdon County, N.J., by Nickle and W.W. Metterhouse of NJDA. This release apparently represents the first release of an entomophagous nematode in the United States (Drea et al. 1977, Nickle 1978*b*, Balaam 1978).

In 1975, 193 juvenile *H. "albicans"* were collected in Austria and shipped to the United States. The Nematology Laboratory received 119 and NJDA received 72 of the living specimens forwarded from quarantine. No releases were made in 1975, and all specimens eventually died in the laboratory. Some of those received at Beltsville successfully overwintered

and molted to the adult stage, but efforts to obtain reproduction in the laboratory failed.

Nematodes identified as *H. "albicans"* were also discovered attacking gypsy moth and several other lepidopterous larvae during ARS studies in Hokkaido, Japan. In 1975, 26 juveniles were sent from Japan to the United States and forwarded to the Nematology Laboratory. In 1976, another 128 juveniles were sent. Living juveniles among the material received in quarantine were divided between the Nematology Laboratory (17), NJDA (52), and the Pennsylvania Bureau of Forestry laboratory at Harrisburg (43). Efforts at Trenton and Beltsville to overwinter the material to obtain adults were largely unsuccessful. Only a few adults were obtained at Beltsville, but there was no reproduction (Nickle 1978 *a*, and Balaam 1978). Of the specimens sent to Pennsylvania, most were received in a weakened condition. Only 17 juveniles were used for a release, on July 19, 1976, near Tower City, Dauphin County, Pa. (Fusco 1978.)

Distribution

In 1977, intensive efforts by ARS workers in Japan resulted in shipment of 663 juvenile nematodes to the United States. The 622 living specimens received were divided between the Beltsville Nematology Laboratory (434), NJDA (76), and the Northeastern Forest Experiment Station at Hamden, Conn. (112). Efforts made to overwinter the material to obtain viable adults, and eggs, were only partially successful; no egg production has yet been obtained. In addition to Old World records, *H. "albicans"* has also been reported from the New World, e.g., from *Hypsipyla grandella* (Zeller) (Lep.: Pyralidae) in Costa Rica (Nickle and Grijpma 1974) and it was reported collected in Virginia (host unrecorded) by G. Steiner (Chapin 1923). Whether these records represent the same species as the *H. "albicans"* imported as a parasite of the gypsy moth is uncertain. A taxonomic study of the *"albicans* species-complex" is planned to help answer this and other questions concerning the possible existence of several species among the many published records of *H. "albicans"* (Nickle 1978*a*).

Information is also needed concerning the biology and life history of these nematodes, including mode of attack, and on the range of hosts capable of being attacked.

Release and Recovery

Thus, small releases of both the Austrian and Japanese nematodes have been made in the New Jersey and Pennsylvania. Collections of gypsy moth larvae have been made each year since release at both the 1974 New Jersey and 1976 Pennsylvania release sites. No nematodes have been found in any of the several thousand larvae collected. (Balaam 1978, and Fusco 1978). It would appear that *H. "albicans"* has not become established in the United States.

Alternate Host Studies

Robert C. Hedlund and Robert F. W. Schroder

The gypsy moth, *Lymantria dispar* (L.), is a univoltine species that is normally active from late April through July. Only the egg stage is present during the remainder of the year. Larval and pupal parasites of this pest must either be adapted to this short period of activity or be able to utilize other hosts. The lack of suitable alternate hosts has been given as the reason for the failure of some species of exotic parasites to establish in the Northeastern United States. Notable among this group are *Apanteles liparidis* (Bouché) and *A. porthetriae* Muesebeck (Burgess and Crossman 1929). These two species are considered important regulating agents of the gypsy moth in their native areas of distribution (Muesebeck 1928, Burgess and Crossman 1929, Vasić 1971).

Studies of alternate hosts of parasites that are established or that have been recently imported have been conducted in the hope of increasing their distribution or of finding adequate alternate host on which they might become established.

Alternate host studies were conducted by the Bureau of Forestry, Pennsylvania Department of Environmental Resources; the Division of Plant Industry, NJDA; the Department of Entomology and Applied Ecology, University of Delaware; and the

Beneficial Insect Introduction Laboratory, ARS, in Beltsville, Md.

Pennsylvania

Gypsy moth parasites that have been recovered from six alternate hosts in laboratory studies conducted by the Bureau of Forestry of the Pennsylvania Department of Environmental Resources are shown in table 6.1–18. Of particular interest is the successful recovery of *A. liparidis* from the tussock moth *Dasychira obliquata*. Many other parasitization and rearing attempts were unsuccessful (Fusco 1978).

New Jersey

The laboratory studies conducted by NJDA are summarized in table 6.1–19. Of particular interest are the successful recoveries of *A. liparidis* and *A. porthetriae* from the eastern tent caterpillar, *Malacosoma americanum*. According to Chianese (1978), the test placed several hundred adult parasites with a few tent caterpillars in a cage that had previously held gypsy moths.

In addition to the laboratory rearing, two parasites were recovered from alternate hosts in the field (Metterhouse 1978). *Brachymeria intermedia* was recovered from the fruit-tree leaf roller, *Archips*

Table 6.1–18. — *Results of laboratory host range studies of imported parasites conducted by the Pennsylvania Bureau of Forestry*

Alternate host	Parasite reared
Fall webworm, *Hyphantria cunea* (Drury)	*Exorista larvarum*
	Palexorista disparis
Symmerista canicosta Franclemont	*P. disparis*
Variable oakleaf caterpillar, *Heterocampa manteo* (Doubleday)	*E. larvarum*
Forest tent caterpillar, *Malacosoma disstria* Hübner	*Exorista segregata*
Fall cankerworm, *Alsophila pometaria* (Harris)	*E. segregata*
Dasychira obliquata (Grote & Robinson)	*Apanteles liparidis*

Source: Fusco 1978.

Table 6.1-19.—*Results of laboratory host range studies of imported parasites conducted by* NJDA[1]

	Apanteles liparidis	Apanteles melanoscelus	Apanteles porthetriae	Blondelia nigripes	Brachymeria intermedia	Coccygomimus instigator	Coccygomimus turionellae	Coccygomimus turionellae moraguesi	Coccygomimus disparis	Exorista larvarum	Exorista rossica	Exorista segregata	Meteorus pulchricornis	Palexorista inconspicua	Palexorista disparis	Rogas indiscretus
Bagworm, *Thyridopteryx ephemeraeformis* (Haworth)	—	—	—	—	—	x	x	x	0	—	—	—	—	x	x	—
Cabbage looper, *Trichoplusia ni* (Hübner)	0	0	0	—	x	—	x	—	—	0	—	x	—	—	—	—
Eastern tent caterpillar, *Malacosoma americanum* (F.)	x	x	x	—	x	x	x	x	x	x	x	x	—	x	x	—
Fall cankerworm, *Alsophila pometaria* (Harris)	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—
Fall webworm, *Hyphantria cunea* (Drury)	0	0	0	—	x	x	x	x	0	x	—	—	x	x	x	—
Forest tent caterpiller, *Malacosoma disstria* (Hübner)	—	—	—	—	—	—	—	—	—	x	x	x	—	—	—	—
Fruittree leafroller, *Archips argyrospilus* (Walker)	—	—	—	—	x	—	—	—	—	—	—	—	—	—	—	—
Orangestriped oakworm, *Anisota senatoria* (J. E. Smith)	0	0	0	—	0	x	x	x	—	0	—	—	—	x	x	—
Mimosa webworm, *Homadaula anisocentra* Meyrick	0	0	0	—	—	0	0	0	0	—	—	—	—	0	0	—
Saltmarsh caterpillar, *Estigmene acrea* (Drury)	0	0	0	—	x	x	x	x	x	x	x	x	—	x	x	—
Sycamore tussock moth, *Halisidota harrisii* Walsh	0	0	0	—	x	0	x	0	0	0	0	—	—	0	x	—
Tomato hornworm, *Manduca quinquemaculata* (Haworth)	—	—	—	—	—	—	—	—	—	0	0	0	—	—	—	—
Birch sawfly, *Arge pectoralis* (Leach)	0	0	0	—	—	—	0	0	—	0	—	—	—	0	0	—
Uglynest caterpillar, *Archips cerasivoranus* (Fitch)	0	0	0	—	—	—	—	—	—	—	—	—	—	x	x	—
Datana angusi Grote & Robinson	0	0	0	x	—	x	x	x	—	—	—	—	—	0	—	—
Greater wax moth, *Galleria mellonella* (L.)	0	0	0	x	x	x	x	x	x	x	x	x	x	x	x	0
Imported cabbageworm, *Pieris rapae* (L.)	0	0	0	—	x	x	x	x	x	0	0	x	—	x	x	—

[1]0 = negative parasite recovery; x = positive parasite recovery; — = no tests conducted.

Source: Metterhouse 1978.

argyrospilus. Compsilura concinnata was recovered from the field-collected alfalfa caterpillar, *Colias eurytheme* Biosduval; cabbage looper, *Trichoplusia ni; Orthosia rubrescens* (Walker); walnut caterpillar, *Datana integerrima* Grote & Robinson; and imported cabbageworm, *Pieris rapae.*

University of Delaware

Studies at the University of Delaware were concerned only with finding alternate hosts of *A. liparidis* and *A. porthetriae* among the Lepidoptera native to the Delmarva peninsula. Larvae from 20 families of Lepidoptera were collected. Forty-five species were exposed to *A. porthetriae* and 53 species to *A. liparidis* (Raffa 1976). The methods used in these laboratory tests have been detailed by Raffa (1977). No *A. liparidis* were reared from any of the species exposed. *A. porthetriae* was recovered from the whitemarked tussock moth, *Orgyia leucostigma* (Smith); the fall webworm, *Hyphantria cunea;* and the yellow woollybear, *Diacrisia virginica* (F.) (Raffa 1977). These studies are continuing, and Russin (1978) has recently recovered an *Apanteles* sp., possibly *liparidis* (determined by P.M. Marsh, Systematic Entomology Laboratory, ARS), from an unidentified geometrid (probably the elm spanworm, *Ennomos subsignarius* (Hübner) or *Phigalia titea* (Cramer)). This was a field-collected larva exposed to *A. liparidis* in the laboratory.

Beneficial Insect Introduction Laboratory

The search for alternate host of polyphagous parasites of the gypsy moth by Beneficial Insect Introduction Laboratory personnel was initiated in 1974 in the western section of Maryland and southwestern Pennsylvania. The search was conducted in the expected path of the southern movement of the gypsy moth. Releases of parasites were made by States bordering the gypsy moth infestation (for example, Virginia, West Virginia, North Carolina) in areas where prospective hosts were often unknown. It was felt that it would be more beneficial to release polyphagous parasites in areas where the insect complex was already known. It is only after the

insect complex is identified and their life cycles known that one can release the most suitable parasites and expect to recover the host and parasites. Thus, the purpose of the work conducted by the laboratory in Maryland and Pennsylvania was to collect and identify forest insects and their associated parasites from areas expected to be invaded soon by the gypsy moth.

Twenty permanent woodlot sites were established, and insects collected from burlap bands at these sites were held in the laboratory for recovery of parasites. Two known parasites of the gypsy moth were recovered. *Compsilura concinnata* was collected at the following sites: Fort Loudon and McConnellsburg, Franklin County, and Caledonia, Adams County, Pa.; and Hancock, Washington County, Md. The hosts of these parasites are unknown except for a *Catocala* sp. (Lepidoptera: Noctuidae) collected at the Caledonia site. *Apanteles melanoscelus* was collected from burlap bands near Westminster, Carroll County, Md.; the host could not be determined. A paper describing the details of these survey studies will be prepared following collection of additional data in 1978.

Several releases of exotic *Apanteles* species were made in 1974 against the fall cankerworm, *Alsophila pometaria* and the elm spanworm, *Ennomos subsignarius.* In May 1974, *Apanteles porthetriae* and *A. liparidis* were released in a fall cankerworm outbreak area on Bull Run Mountain, Prince William County, and at Skyline Drive, Page County, Va. *Apanteles* spp. was not recovered from fall cankerworms collected either the same year or in subsequent collections the next 2 years. *Apanteles porthetriae* and *A. liparidis* were also released against elm spanworm near Blue Ridge Summit, Washington County, Md., in summer 1974. They were not recovered the same year or the following year from this site or from adjacent woodlots where elm spanworm larvae were collected.

The close proximity of some of the laboratory study sites to those set up by Dr. Coffman of the West Virginia Department of Agriculture allowed for the comparison of collection data. Results indicated that *Dasychira meridionalis* (Barnes &

McDunnough) (Lepidoptera: Lymantriidae) and *Polia latex* (Guenée) (Lepidoptera: Noctuidae) were the most abundant species collected in the woodlot surveys. *D. meridionalis* overwinter as larvae on the underside of branches, making the species a prospective alternate host for *A. liparidis*. Because *P. latex* was very abundant and readily available, it was chosen for testing as a potential host for *A. liparidis*. There was little information available on the life cycle of *P. latex*, especially as to whether it overwintered in the area as pupae or larvae. Cage studies were conducted in late 1977 in cooperation with Coffman, near Romney, Hampshire County, W. Va. Studies were run concurrently with this project to determine the suitability of *D. meridionalis* and *P. latex* as potential alternate hosts of *A. liparidis*. These studies are not yet complete.

Discussion

Apanteles liparidis and *A. porthetriae* are the two most abundant parasites of the gypsy moth in Europe and Asia that have not been established in the United States. They attack the early larval to midlarval stages, the stages least attacked by parasites in the United States; the desirability of establishing these species is therefore obvious. The failure of establishment attempts is believed to be due to a lack of suitable alternate hosts. The alternate hosts of *A. porthetriae* (if indeed there are any) are unknown (Vasić 1971). There are four reported alternate hosts of *A. liparidis*: *Dendrolimus pini* (L.) in Europe, *D. spectabilis* Butler in Japan (Burgess & Crossman 1929), *D. sibiricus* Tschv. in the U.S.S.R., and *D. albolineatus* (Mats.) in the U.S.S.R. and Japan (Telenga 1955). The genus *Dendrolimus* has not been reported from North America (McDunnough 1938).

A similar problem was encountered with the fall webworm, *Hyphantria cunea* (Drury), after it was introduced into Yugoslavia. Several unsuccessful attempts were made at biological control. Pschorn-Walcher (1977) stated that most of the parasite species introduced from the United States require alternate hosts, and this may have been a reason why none of them became established in Yugoslavia.

There are no known similar situations involving alternate host problems that have resulted in successful establishments, but this should not necessarily discourage future efforts. As the gypsy moth continues to move into new areas of the United States, the diversity of native potential host species may produce a species capable of acting as an alternate host to one or both of these potentially effective *Apanteles* species. Releases should therefore be continued as the gypsy moth spreads. In addition, a thorough study of the life cycles of these parasites should be undertaken. Their potential effectiveness justifies the expense of determining all alternate host, climatological, and behavioral requirements.

Intensive Laboratory and Field Evaluations of Individual Species

Blepharipa pratensis (*Meigen*) (*Diptera: Tachinidae*)
Paul A. Godwin and Thomas M. ODell

Introduction

The biological control of the gypsy moth has been attempted by the introduction and establishment of a number of parasites and predators. Of the 50 or so parasites introduced, 10 are considered to be established. In no case has the outcome of these releases been predictable, nor are the reasons for success or failure known (Hoy 1976).

Most gypsy moth researchers have measured the degree of success of a particular parasite species in terms of the number of gypsy moths killed. This is understandable, because the emphasis has been placed on biological control rather than on the survival success of the parasite. This has not led, however, to an understanding of the underlying processes that control the parasite population system necessary for the development of predictive models.

Significant advances have been made in the understanding and management of pest-insect populations through studies of an insect's population dynamics. A similar approach should succeed for a parasite population.

Blepharipa pratensis (Meigen) (fig. 6.1–6) is a tachinid fly native to the Palaearctic region. There it is considered to be the most consistently dominant parasite of the gypsy moth (Herting 1960a, Pschorn Walcher 1974).

In 1905, it was among the first parasites introduced into North America, by the U.S. Department of Agriculture, to control the gypsy moth. This species was released in 1906–07 and found in 1910–11 in circumstances that suggested that it had become established. Additional importations and releases were also made. In North America, the fly is found throughout the general area of gypsy moth infestation, which has come about by natural dispersal and the transfer of the parasite from older infestations to newer ones. As in Europe, it is considered in the United States to be the most important parasite of the gypsy moth (Angalet 1974).

Because completion of a population study could not reasonably be expected within the the time limits of the gypsy moth research program, the general objective of this research was to determine, using population-study techniques, whether there was an aspect of the life history or behavior of this fly that would lend itself to manipulation so that the species could be managed as a biological control agent. In this respect it was a feasibility study and not an attempt to develop a control method. Four objectives were sought:

1. To develop methods of counting the fly in its various life stages.
2. To identify major mortality factors, required for the development of life tables and, subsequently, for the development of population models. They are also essential, now, to the interpretation of "apparent percent parasitism" in terms of parasite survival and gypsy moth mortality.
3. To develop methods of rearing the fly in the laboratory.
4. To describe the fly's reproductive behavior.

The immediate reason for conducting research with these last two objectives was to obtain a source of flies for research. In the long run, manipulation of parasite reproductive behavior might be a practical pest management option, since the release of laboratory-raised parasites has been *the* management scheme for most other parasites.

A discussion of research results will be presented under six major headings—adult, egg, larva, and pupa bionomics, field populations, and laboratory culture—followed by a summary.

Adult Bionomics

Emergence

Blepharipa pratensis overwinters as a pupa within a puparium. In the spring, the adult escapes from the puparium by inflating the eversible sac (ptilinum) of the frons and rupturing the anterior end of the puparium (Metcalf et al. 1951). The callow adult pulls its way out of the puparium and makes its way through the soil and duff in which it was buried. Once in the clear, the adult crawls to a place where its wings can unfurl and dry. After 1 to 2 hours, the fly has its normal coloration and flight is possible. Sabrosky and Reardon (1976) have described the adult in detail.

The emergence of adult *B. pratensis* provides the first opportunity to count and estimate the endemic population of this parasite. To do this, a trap was designed that would catch flies as they crawled from the forest litter, which would represent a sampling area of 0.093 m² of forest floor (fig. 6.1–7).
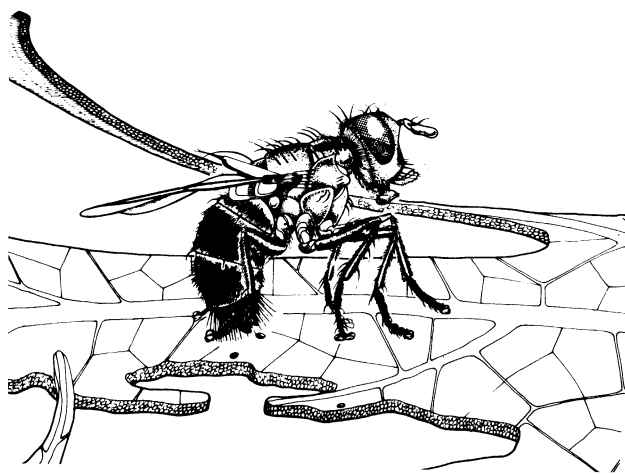
Figure 6.1–6.—*Adult* Blepharipa pratensis (*Meigen*).

Since the *B. pratensis* maggot emerges from the host pupa, and pupariates soon after, the location of the puparium, and thus the site of adult emergence, is directly influenced by the location of the host pupa-
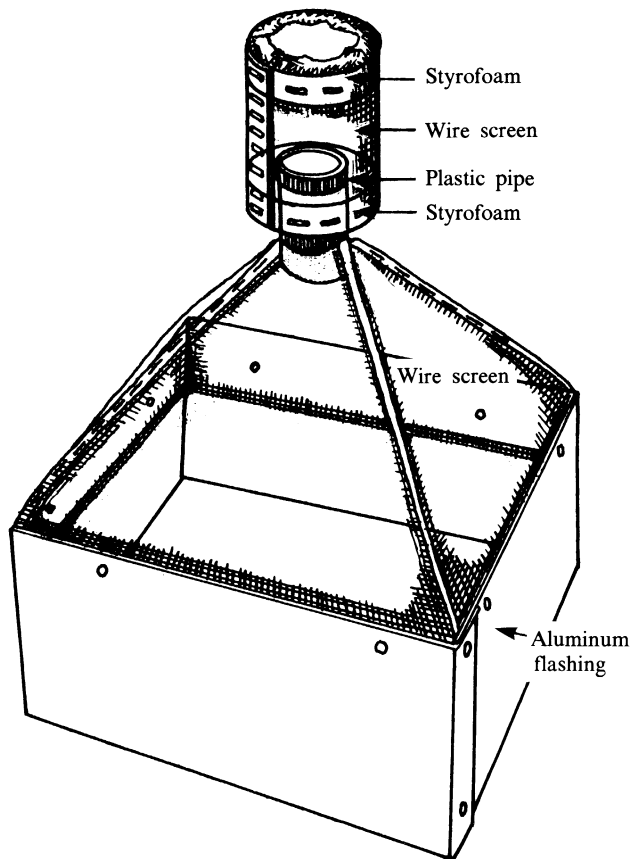
Figure 6.1-7.—*Trap for catching emerging* Blepharipa pratensis (*Meigen*) *adults.*

tion site. Considering this, the placement of traps for catching emerging flies should relate, in some way, to the distribution of gypsy moth pupae. Unfortunately, techniques to estimate this were not available. Consequently, in 1975 traps were arrayed in a grid on the forest floor in Centre County, Pa. In a 7432.24 m^2 plot, 480 traps were placed at 3.04-m intervals along 122-m transect lines spaced 4.87 m apart. Each respective trap site was described according to the parameters listed in table 6.1-20.

The Centre County area was selected because many *B. pratensis* adults had been caught in an adjacent area in 1974. From May 19–27, 1975, 98 *B. pratensis* adults were caught in 74 traps. The occurrence of particular site characteristics for these 74 traps was compared to the occurrence of these characters for all traps; because of the relatively small sample size, statistical analysis was not feasible. General observations were:

—Location of traps relative to tree species was the most important site characteristic associated with *B. pratensis* catches, and trap sites located under crowns of northern red oak (*Quercus rubra* L.) or sweet birch (*Betula lenta* L.) were more likely to have emerging *B. pratensis*, while trap sites under red maple (*Acer rubrum* L.) were least likely.

—The placement of traps relative to DBH, crown class, trap to tree, and trap to crown categories did not appear to be directly associated with fly catch but may indirectly affect fly catch depending on tree species.

—Fly catch did not seem to be associated with the occurrence of particular ground cover, shrubbery, or placement of trap relative to bole sweep.

Table 6.1-20.—*Trap site parameters for identifying overwintering site of* Blepharipa pratensis

Distance from tree stem	d.b.h. category	Tree crown placement	Bole sweep	Crown class	Ground cover
Fewer than 0.72 m	Fewer than 5.8 cm	One-quarter over trap	No sweep	Dominant	Moss + leaves
.72–1.1 m	5.8–7.6 cm	One-half over trap	Away from trap	Codominant	Moss + rock
1.15–1.4 m	7.7–9.9 cm	Three-quarters over trap	Toward trap	Intermediate	Leaves + moss
1.45–1.75 m	10 cm–12.2 cm	Completely over trap	Over trap	Overtopped	Leaves + rocks
1.8–2.1 m	12.3–15.5 cm	Drip line over trap			Rocks + leaves
21.5–2.5 m	15.6–19.5 cm				
2.55–3.15 m	19.6–24 cm				
	+24.5 cm				

—Fly catch did seem to be associated with the occurrence of certain tree species environments—that is, where two or three trees of particular species occur together in a trap site and each crown partially covered the trap. Trap sites containing two or more sweet birches, or a combination of northern red oak and sweet birch, were more likely to have emerging *B. pratensis*, while trap sites containing two or more red maple or a combination of red maple and another species were least likely.

Trap site analysis in 1975 provided some clues for increasing the efficiency of trap placement in this site but not necessarily in other sites, since tree species vary from site to site. Finally, the variation among sampling units (trap sites) indicated that more units were required if the plot area remained the same. Subsequently, 500 traps were arrayed in 0.5 ha population-study areas in 1976 (see section on Field Populations).

In the Northeast, emergence of *B. pratensis* begins in the first 2 weeks of May at approximately the same time of gypsy moth egg hatch. Clausen (1956) reported that *B. pratensis* (=*B. scutellata*) emerged a week or two before egg hatch was general. In 1973, in Branford, Conn., emergence of *B. pratensis* began on May 14, which coincided with gypsy moth egg hatch in the area.

According to records covering emergence of *B. pratensis* in Eastford, Conn. (1977), Washington Township, Pa. (1975, 1976, 1977), and New Lisbon, N.J. (1976, 1977) (see section on Field Populations), emergence began in the first week of May and continued through the first week of June. Emergence in New Lisbon occurred 5 to 7 days earlier than in the other areas and, in general, coincided with *B. pratensis* in Morristown, N.J. (Dalton 1976); that is, emergence began on May 6, peaked around May 19, and ceased on June 1.

The seasonal pattern of emergence is different for males and females. Males begin emerging first, with peak emergence occurring approximately 5 days before peak female emergence. This places the majority of males in the field during peak female emergence. Laboratory and field cage studies indicate that 80–90 percent of the adults emerging on any particular day do so between 7:00 a.m. and noon. In the field, emer-

gence time during the day varied from early morning to midafternoon. Although the effect of temperature on *B. pratensis* emergence was not specifically measured, fluctuating temperature in the field plots appears to be the primary factor regulating the temporal occurrence of emergence.

Mortality

Factors that affect the survival or, conversely, the mortality of *B. pratensis* adults are the most difficult and time consuming to discern and measure. Therefore, attempts to identify and measure these factors in the fly's natural habitat were not made. However, the survival of captive adults was studied, and, where possible, the factors associated with mortality were identified.

Approximately 2 percent ($N=210$) of the *B. pratensis* adults captured in ground traps had morphological deformities, usually crumpled wings. The average longevity of the deformed adult in captivity was 7 days, but because it most likely could not fly, its life span in the natural environment would likely be much less.

The average longevity of females held in plastic-bag cages at normally fluctuating temperatures (not in an environmentally controlled regime) was 12.2 days. However, females that survived to lay eggs (see Oviposition) lived an average of 22 days, while others lived an average of 8.6 days. Males lived an average of 10.8 days.

Most of the flies that died were examined for microorganisms to determine if pathogens were present ($N=416$). Single isolates of *Serratia marcescens, Pseudomonas aeruginosa,* and *P. fluorescens* were found, respectively, in 46.4 percent, 15.6 percent, and 7.5 percent of the 416 dead adults examined and were the predominant species associated with dead adults. *Enterobacter cloacae, E. agglomerans, Bacillus cerea,* and a yeastlike organism were also isolated. No microorganisms were found in 96 (23 percent) of dead adults examined.

S. marcescens, P. aeruginosa, and *P. fluorescens* are considered "borderline pathogens"—that is, given the proper environment they will rapidly proliferate and cause death (Bucher 1960). *S. marcescens* was identified as the microorganism causing widespread

mortality in laboratory colonies of gypsy moth larvae that had been fed *B. pratensis* eggs, but it was not possible to isolate *S. marcescens* from *B. pratensis* eggs. *S. marcescens* is also highly pathogenic for second-instar larvae of the gypsy moth (Podgwaite and Cosenza 1976) and has been identified as the causal organism in disease outbreaks of other laboratory insect populations (Bucher 1960, Steinhaus 1959). King et al. (1975) reported *S. marcescens* as the primary cause of a disease outbreak affecting the laboratory populations of the sugarcane borer, *Diatraea saccharalis* (F.) and its tachinid parasite, *Lixophaga diatraeae* (Townsend). *P. aeruginosa* and *P. fluorescens* have been isolated from the desert locust, *Schistocerca gregaria* Forskal, and several lepidopterous species (Bucher 1960).

Adult flies in captivity tend to rest in relatively concealed places, and it is suspected that this may also be true in natural environments. In field cages spiders and ants have been observed eating resting flies. Forbush and Fernald (1896) suggest that tachina in general are probably eaten by birds but offered no specific evidence. During this study, birds were not observed attacking and feeding on *B. pratensis,* but their relatively large size would lend credence to this possibility.

Feeding

Although feeding by adult Diptera is generally supplemental to that of the larva, circumstances may exist that would cause the insect to require additional nutritional support (adults need water to replace that lost in flight, carbohydrates to provide energy for their flight, and extra protein for egg production (Oldroyd 1966). As an adult, *B. pratensis* is relatively long lived (up to 38 days in captivity) and is an active flier. The female produces an unusually large number of eggs, up to 4,820 (ODell and Godwin 1978). Thus, it would be reasonable to expect an adult to require additional sources of energy.

Tachinids in general feed on the honeydew secreted by other insects and on various plant secretions, and some feed at plant blossoms (Clausen 1972). During this study of *B. pratensis,* the feeding behavior or dietary requirements of adults were not specifically inves-

tigated, but casual observations were made whenever and wherever possible. The following summarizes these observations.

Pollen (Hilleman 1963) and aphid honeydew (Way 1963) provide a good source of protein for reproduction and carbohydrates for maintenance of flight capability and could conceivably be the major energy resources utilized by *B. pratensis. B. pratensis* adults were offered black oak (*Quercus velutina* L.) leaves covered with pollen from white pine (*Pinus strobus* L.); they readily fed on the pollen, creating feeding trails as they moped across leaves. Red maple leaves with aphid honeydew on both upper and lower surfaces (aphids were removed) were also offered to adult flies, which were immediately attracted to the leaves and fed readily on contact with the honeydew.

During this period a wide range of flowering plants ($N = 17$) in the area were monitored on an irregular schedule to determine if the fly visited and fed on pollen in the flower; *B. pratensis* was not observed on any of them.

Mating

B. pratensis mated (fig. 6.1-8) readily in plastic-bag cages (ODell and Godwin 1978); mating frequency is increased by maintaining cages outside in indirect sunlight at temperatures between 21° to 32° C (cage
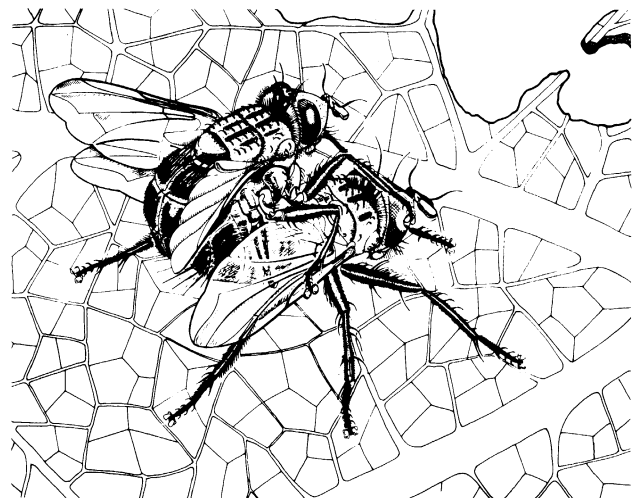
Figure 6.1-8.—*Mating pair of* B. pratensis.

temperature was not measured). Mating occurred most often in the afternoon with peak encounters and copulation occurring around 2:00 p.m. Females ($N=20$) mated an average of two times (range 1–7), while males were able to fertilize up to five females. The average time in copulation for first encounters (20 pairs) was 111 minutes, with a minimum of 30 minutes and a maximum of 280 minutes. Most females mated in the first 48 hours after they eclosed and often before they lost their callow appearance. Older (72 hours) unmated females do not appear to be as receptive.

Males appear to be activated by movement and will fly at and strike other flies in flight. If this happens to be a female, mating will be attempted. We have observed males inadvertently coming in contact with dead females and attempting to mate with the cadaver. These observations indicate the presence of a sex recognition pheromone on the cuticle of the female, similar to that described for the tsetse fly, *Glossina morsitans* (Westwood) (Langley et al. 1975), and the female stable fly, *Stomoxys calcitrans* (L.) (Harris et al. 1976).

In May 1977, 4 to 5 days after male eclosion began, male and female *B. pratensis* were watched and then collected as they flitted and rested at the lower shrub level in the forest study plot in Washington Township, Pa. During the collection periods mating pairs were also observed and collected. Apparently the male, emerging first, maintains a position near the area of probable female emergence, thus increasing the chance of encounter and taking advantage of the receptiveness of newly eclosed females. If mate finding is primarily by visual perception of a moving female, the adverse effects of naturally occurring low population densities on mating frequency may be overcome by this behavior.

Dispersal

The factors effecting dispersal of *B. pratensis* have not been specifically studied, and miscellaneous observations are scarce. Tachinids are known to be strong fliers, and there is no reason to suspect that *B. pratensis* does not fit this general description.

Since *B. pratensis* was first introduced in New England in 1905, thousands of these flies from domestic stock have been distributed, and it is now well established throughout the northeastern range of the gypsy moth (Sabrosky and Reardon 1976). Outlying gypsy moth infestations within the quarantine area and in new infestations in States outside the quarantine area such as Michigan and Wisconsin have apparently been started by the inadvertent transport of gypsy moth pupae and egg masses into these areas. Since a *B. pratensis* larva spends at least 7 days in the gypsy moth pupa, dispersal of this parasite may be affected in the same way. Thus, considering the extent of all possible mechanical distribution of *B. pratensis*, intentional or not, the dispersal capability of *B. pratensis* via flight will continue to be difficult to assess. Nevertheless Burgess and Crossman (1929) attributed the recovery of *B. pratensis* (=*Sturmia scutellata*), found 482.8 km from the nearest source population, to flight dispersal.

Very little is known about the factors initiating flight and dispersal. Newly emerged males and females tend to remain near the forest floor and appear to spend as much time resting and preening their wings as they do flying. Since mating occurs primarily soon after female emergence, it would seem advantageous to the species for the male to remain in close proximity to the emergence site, in lieu of dispersal. On the other hand, the female, once mated, has approximately 2 weeks before oviposition begins and most likely uses the time to search for an oviposition site, and dispersal may occur during this preoviposition period (Clausen 1972). However, once egg laying begins, the tendency for dispersal diminishes. Experiments in field cages support this generalization: when flies are released in a $1.8 \times 1.8 \times 2.44$ m screen cage containing clusters of oak leaves, fecund *B. pratensis* fly directly to the leaves and begin to lay eggs, while nonlaying females fly to the top or sides of the cages as if trying to escape.

Host Finding

B. pratensis is one of the tachinids that deposits microtype eggs on the foliage of the host's food plant (Clausen 1972). Townsend (1908) first noted this behavior and suggested that the female was attracted to trees where gypsy moth larvae were most abun-

dant. From towers and platforms erected around three large black oaks, the egg-laying behavior of *B. pratensis* was observed and leaf samples were collected to determine where eggs were laid. Approximately 84 percent of the eggs were found on leaves from the upper crown, where gypsy moth larvae are most likely to feed. Because oviposition occurred in the afternoon, however, it would appear that most of the eggs found were put there during a time of day (afternoon) when the host is least likely to be feeding (Leonard 1970). This indicates that the selection of the oviposition site would depend on stimuli other than the presence of the host.

Many parasites use physical and chemical cues to locate their hosts (Vinson 1976). *Zenilla libatrix* (Panzer), another tachinid parasite of the gypsy moth, and *Leschenaultia exul* (Townsend), a tachinid parasite of the eastern and forest tent caterpillars *Malacosoma americanum* (Fab.) and *M. disstria* (Hbn.), apparently oviposit their microtype eggs in response to contact with the damaged edges of leaves fed on by their hosts (Dowden 1934, Bess 1936). *B. pratensis* responds similarly to damaged leaves; the fly flits from leaf to leaf, lapping and/or just examining the leaf surface with its proboscis. Encounter with a freshly damaged leaf edge by the front tarsi causes immediate oviposturing; eggs are not always laid, however.

Experiments in the laboratory using flies confined in plastic bag cages showed that the presence of damaged leaves artificially cut or eaten by gypsy moth larvae affected where eggs were laid. Eggs were about equally distributed between bag and leaf in cages with damaged leaves, but in cages without damaged leaves, 80 percent of the eggs were laid on the plastic bag.

Egg distribution was not apparently affected by whether or not the leaf was eaten or cut, but flies caged with eaten leaves laid 50 percent more eggs than those with cut leaves. There was no apparent difference between the total number of eggs laid by females caged with cut leaves and those caged with undamaged leaves, and the presence or absence of the host larva did not affect oviposition.

Silk deposited by the host has seldom been found on leaves harboring *B. pratensis* eggs, and feeding by other lepidoptera does not appear to elicit the same oviposition behavior. The evidence so far indicates that host location by *B. pratensis* consists of at least two responses—a plant mediated chemotactic response that appears to be the sign stimulus for host location, and a host-specific chemotactic response that stimulates oviposition.

Oviposition

The natural, seasonal occurrence of oviposition closely paralleled the pattern of oviposition of captured *B. pratensis* held outside. The average time from mating to initial oviposition of flies held in cages outside was 16.8 days ($N=66$), with peak oviposition occurring approximately 4 weeks later. In the field, we did not observe *B. pratensis* laying eggs until the majority of gypsy moth larvae were in the late third instar or early fourth instar. In the population study areas this was during the last 2 weeks of May. Peak egg laying occurred during the second and third weeks of June, when the majority of gypsy moth larvae were in the fifth and sixth stages.

Egg Bionomics

Eggs are ovoid, slightly depressed dorso-ventrally, 0.16 to 0.20 mm wide at their widest part, and 0.25 mm long. The chorion is black with raised reticulations except for a narrow longitudinal band on the ventor, which is less pigmented and somewhat thinner. Howard and Fiske (1911) published an illustration of the egg and Thompson (1924) described and gave egg dimensions. As an egg is laid, a small amount of gelatinous material is also deposited to secure the egg to the substrate. This hardens rapidly but may become gelatinous again in the presence of free water or high humidity. Infection is initiated when a host larva swallows an egg during feeding.

Viable eggs are fully embryonated upon deposition. Viability can be determined by removing the chorion of the egg and looking for the pharyngeal hooks; when an egg is gently probed, a living maggot will retract its pharyngeal hooks. The vitelline membrane of infertile eggs contains only a milky fluid. It was found that 10 eggs sampled daily from eggs laid the previous day (from each female) gave a reliable

estimate of viability. Eggs laid by flies collected with nets in May 1977 near the Pennsylvania population plot (see Field Populations) had an average viability of 87 percent ($N=63$). In 1975, the fertility of eggs laid by females emerging in cages and held outside with males was 84 percent ($N=126$); the viability of eggs from females held outside was only 54 percent ($N=77$).

Most eggs of *B. pratensis* were laid on the lower surface of the leaf. Godwin and ODell (1973) found that 81 percent of the 206 eggs found on 165 leaves were laid on the lower surface. Microtype eggs laid on the upper leaf surface lose water and their viability more quickly than those on the underside (Bess 1936).

The amount of time eggs remain viable while on leaves was tested by sampling eggs of known age and feeding them to host larvae in the laboratory. Eggs were collected each day for 7 days from the top of a tree and fed individually to 112 host larvae. Eggs remained viable at least 7 days. Eggs of *Leschenaultia exul* remained viable for at least 20 days (Bess 1936), so it is expected that *B. pratensis* eggs are similar. In the laboratory, eggs can be refrigerated at 0° to 2° C for up to 4 weeks without significant loss of viability. Further investigation is needed of how long eggs remain viable in the tree.

Larva Bionomics

A fertile egg laid by *B. pratensis* is essentially a fully developed maggot surrounded by the vitelline membrane and chorion. The chorion is a rather tough, semirigid material. When pressure is applied with a needle to the dorsal or lateral surface of an egg, the maggot, enclosed in the membrane, pops out of the chorion, undamaged more often than not. This characteristic of the chorion probably helps the maggot escape the crushing action of the gypsy moth's mandibles. It doesn't always help, however; first-instar larvae cannot ingest an egg whole. More than 90 percent of the eggs eaten by second-instar larvae and 60 percent of those eaten by third-stage larvae are fatally crushed. Among fourth-, fifth-, and sixth-instar larvae an average of 62 percent survived. The range was from 89 percent in a group fed to fourth-instar larvae to a low of 44 percent fed to fifth-instar larvae.

Kathleen Stone Shields (1976) has described in detail the sequence of infection. After ingestion, the maggot emerges before the egg reaches the midgut. It then bores through the gut wall, often in as little as 35 minutes, and enters into the hemocoel, where it moves about freely. After a period of from 4 to 20 hours, the maggot enters one of the longitudinal intersegmental muscles of abdominal segments two through six. The presence of the maggot in the muscle induces a tumorlike growth characterized by a muscle cell proliferation and cellular and nuclear hypertrophy (Howard and Fiske 1911, Shields 1976).

Thompson (1924) has described the first-instar maggot. It remains in the muscle in instar one until the onset of host pupation. Consequently, maggot development time depends on the age of the host when the egg is ingested. It ranges from 36 days for those eaten by second-instar larvae to 17 days for those eaten by fourth-instar larvae. During this period, the maggots grow very slowly and weigh less than 0.1 mg through the host's fourth instar. Starting in the host's fifth instar, maggots in male larvae grow at the rate of 0.6 mg a day. Those in female larvae grow at the rate of 0.3 mg a day through the fifth and sixth instars.

With the onset of host pupation (the prepupal stage lasts about 24 hours), the maggot molts to instar two and, although it remains in the muscle, begins to grow rapidly. When pupation takes place the maggot leaves the muscle and forms a respiratory funnel directly to a pupal spiracle, usually within 18 hours. The maggot molts to instar three 3 to 4 days later. The third-instar larva has been described by Peterson (1951). Growth in this period can be described by the equation:

$$Y = 2590.4676 + 846.3503X - 91.1527X^2 + 3.4401X^3$$

where:

   Y = maggot weight in milligrams, and
   X = number of days elapsed from egg hatch.

Not all maggots that hatch survive to the end of the third instar, however. The chances of a maggot surviving increase as the age at which the host larva swallows the egg increases. Only 1.75 percent of the maggots that hatch in second-instar gypsy moth lar-

vae survive. Survival increases to 15 percent for those eaten by third-instar larvae. Survival rates for those eaten by the fourth, fifth, and sixth instars were 49, 52, and 77 percent, respectively.

A number of factors that contribute to maggot mortality have been identified. Superparasitism is common in field populations where *pratensis* egg density is high. For example, in an area where egg density was 39 eggs per square meter of leaf area, 72 percent of the infected fifth- and sixth-instar larvae were superparasitized. As many as 17 maggots have been dissected from a single gypsy moth larva. But the survival of more than one maggot to the puparium stage under these conditions is a rare event. When two eggs are fed to larvae in the laboratory, both individuals survived to pupariate only 8.76 ± 1 percent of the time.

Another source of mortality is interspecific competition with other parasites. *Parasetigena silvestris* (R.-D.) may reduce *B. pratensis* survival by as much as 60 percent. The degree to which survival is reduced depends on the stage of the host and the sequence in which infection by the two parasites takes place.

On the other hand, *Brachymeria intermedia* (Nees), a hymenopterous parasite that attacks gypsy moth pupae, has little or no effect on *B. pratensis* survival. But *Brachymeria compsilurae* (Cwfd.), a closely related species, is a parasite of *B. pratensis*. It infects the maggot while it is in the gypsy moth pupa but does not kill it until the prepupal or pupal stage. Its impact will be discussed later in the section on Pupa Bionomics.

A common hazard for all parasites is the death of the host before the parasite can complete development. *B. pratensis* is no exception, but infection by *B. pratensis* appears to increase the probability that the host will die of causes, usually unknown, other than myiasis. The degree to which *pratensis* infection increases premature death of the host depends on the age of the host when infected. These deaths range from 67 percent among larvae infected in the third instar to 12 percent among sixth-instar larvae. Multiparasitism and superparasitism also increase the number of premature larval deaths.

Parasitization by *B. pratensis* increases the probability that the host will be eaten by a predator. Smith (1978) has observed that the white-footed mouse

selects *pratensis*-infected pupae over noninfected pupae when given a choice.

About 4 days after pupation the host dies, and 7 days after this the maggot leaves the carcass. There is a marked daily periodicity to maggots leaving the host. Most emergence takes place before 9:00 a.m., with the peak emergence at about 7:30 a.m. There is a second, much lower peak of emergence near 4:00 p.m. Seasonal emergence of maggots follows the rate of host pupation very closely (fig. 6.1–9).

Although most maggots do not complete development until the host pupates, an average of 11.76 ± 1 percent complete development while the host is still a larva. In these cases, emergence of the maggot follows the death of the larva by about 3 days. What triggers this precocious development, however, is not known.

Pupa Bionomics

Growth and Development

After issuing from the host pupa, the full grown *B. pratensis* maggot crawls into the duff, usually to the first layer of soil, and forms its puparium; that is, when larvae of cyclorrhaphous flies are fully grown, the epidermis adds to the existing cuticle a substance that slowly hardens and darkens to form a shell or puparium (Fraenkel and Bhaskaran 1973). For *B. pratensis*, this tanning process takes approximately 24 hours to complete and is called pupariation. In the
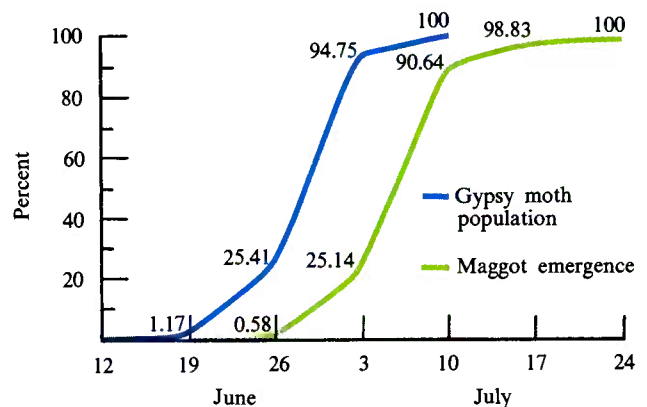
Figure 6.1–9.—*Seasonal rate of gypsy moth pupation and rate of emergence of* B. pratensis *maggots from host pupae in a New Jersey oak forest, 1976.*

stage between completion of the white puparium and the following molt (larval-pupal apolysis), the developing fly should be called the prepupa.

Radiography (ODell et al. 1974) was used to follow larval-pupal apolysis in puparia held at approximately 24° C. In the first 7 days following pupariation, no specific morphological changes were discernable. On day eight, the pupal form could be distinguished. Dissections of these puparia reveal a soft, white prepupa enclosed in a transparent sheath. At 15 to 20 days following pupariation, the pupa has all the morphological appearances of the adult fly, except that the black setae are distinctly apparent against an off-white body integument; the eyes are reddish, the mouthparts and legs are gray to black, and the head and thoracic regions are distinct (phanerocephalic stage, Fraenkel and Bhaskaran 1973). Later dissections, including samples shortly before emergence, showed no obvious morphological advancement. Spermatogenesis is completed 4 to 8 days after pupation.

Mortality

In the early stages of this investigation a number of people were contacted who, at one time or another, had attempted to collect, transport, and release *B. pratensis* as a means of colonizing particular areas in the Northeast and in the Virginias. For the most part these attempts were futile, primarily because of poor emergence of adult *B. pratensis* in the spring. The apparent overwintering mortality caused losses of 50 to 70 percent of the collected puparia. Dalton (1976) showed that handling of puparia during collection and placement significantly reduced adult emergence the following spring but still had 34 percent mortality in no-handling treatments.

The first look at this overwintering mortality in 1973 led to the belief that much of this mortality occurred during the period between formation of the puparium and development of the pupa. For example, the contents of 45 percent of the *B. pratensis* puparia examined (N=64) for pupae 8 days after pupariation were either liquified or dried up. Subsequently, in 1974, 3,464 puparia, recovered from 12,751 gypsy moth pupae collected from sites in

Connecticut, New York, New Jersey and Pennsylvania, were radiographed to determine if *B. pratensis* had pupated.

Depending on collection site, the results indicated that 29 to 56 percent of the *B. pratensis* died between pupariation and pupation. Microbiological analysis of 900 of these undeveloped specimens gave the following results: No microorganisms were found in 50 percent of the puparia; in puparia containing microorganisms the number of species per host ranged from one to four; there was no significant difference in the occurrence of parasite microorganisms based on collection site; and, of the microorganisms identified (table 6.1-21), *Proteus mirabilis* had the highest incidence of occurrence (50 percent of puparia containing microorganisms).

Proteus spp. and in particular *P. mirabilis* and *P. vulgaris* are "potential" pathogens and are pathogenic in several species of Lepidoptera and Orthoptera (Bucher 1960). The frequency of their occurrence in *B. pratensis* puparia suggests that they are lethal pathogens of *B. pratensis* during the period between pupariation and pupation.

Table 6.1-21—*Species of microorganism found in* Blepharipa pratensis *puparia and the host gypsy moth pupae after parasite emergence*

Species
Staphylococcus epidermidis[1]
Staphylococcus aureus[1]
Streptococcus faecalis
Escherichia coli
Enterobacter cloacae
Serratia liquefaciens
Serratia marcescens
Proteus vulgaris
Proteus mirabilis
Proteus morganii
Proteus rettgeri
Pseudomonas aeruginosa
Pseudomonas fluorescens
Pseudomonas cepacia
Pseudomonas putida
Aeromonas sp.[1]

[1]Found only in host. All other species were cultured from parasite puparia and host pupae, but seldom in both host and its respective parasite.

In addition to the autopsy of dead puparia, each gypsy moth pupa that yielded a *B. pratensis* maggot was examined. Although there was no indication that the host contributed directly to parasite mortality via transmission of a pathogen, significant differences were identified in the microorganism species composition between hosts whose parasites failed to survive and those whose parasites survived. This indicates that the microorganisms existing in the host during parasite development may directly affect *B. pratensis* development and survival during the period between pupariation and pupation.

Brachymeria compsilurae (Cwfd.) is a primary parasite of tachinid flies and has been considered a serious threat to the effectiveness of *Compsilura concinnata* (Meig.) and *B. pratensis* (=*scutellata*) (Dowden 1935). *B. compsilurae* attacks the *B. pratensis* maggot while it is in the gypsy moth prepupa or pupa but does not kill the maggot until the maggot has formed its puparium. Using radiography, *B. compsilurae* larvae can be easily distinguished in 6- to 7-day-old *B. pratensis* puparia.

In 1973, *B. compsilurae* adults were collected as they emerged from *C. concinnata* puparia recovered from gypsy moth larvae in Branford, Conn. Peak emergence of the hyperparasite occurred between June 18 and 21. Gypsy moth larval counts indicated that at that time the majority of larvae were in the fifth or sixth instar. Dowden (1935) indicates that *B. compsilurae* may readily shift from one host to another. What has been left unemphasized is the increased potential of the first full generation of *B. compsilurae* emerging from *C. concinnata* to parasitize *B. pratensis* feeding in pupae of the gypsy moth. In essence, the smaller host, *C. concinnata*, provides a substrate that increases the hyperparasite population of *B. pratensis*.

Although *B. compsilurae* is generally considered the dominant hyperparasite of *B. pratensis*, statistics on the number of *B. pratensis* killed are seldom provided. The absence of such data is perhaps indicative of the methods used to obtain *B. pratensis* maggots and puparia. Most collections of *B. pratensis* are made by collecting gypsy moth pupae and holding them until maggots emerge. Depending on the time of collection relative to gypsy moth pupation, this method effectively removes *B. pratensis* from further attack by *B. compsilurae* and biases the percent parasitism statistic.

The first indication of the potential impact of *B. compsilurae* as a hyperparasite of *B. pratensis* came from Tigner (1974), who found that 36 percent of the 881 *B. pratensis* puparia recovered from gypsy moth pupae collected in New York were parasitized by *B. compsilurae*.

Subsequently, in 1974 3,464 *B. pratensis* puparia were recovered from 12,571 gypsy moth pupae collected from five different locations in the Northeast; the proportion of puparia containing *B. compsilurae* ranged from 1 to 30 percent (table 6.1-22).

Although site characteristics relative to the incidence of *B. compsilurae* were not specifically investigated, some comments on these areas are worth noting. The sites with the greatest hyperparasitism were, at the time of collection, open and sunny; the Woodstock, Conn., site (24 percent hyperparasitism) was almost completely defoliated, and the Pennsylvania ridge site (30 percent hyperparasitism) was sparsely covered with low-growing scrub oak. In comparison, all other sites were generally shaded; the Pennsylvania valley site (8 percent hyperparasitism) was located only 1 mi from the ridge site, but was well shaded. The inference here is that *B. compsilurae* may prefer well-lighted, sunny habitats, similar to those

Table 6.1-22.—*Incidence of the hyperparasite* Brachymeria compsilurae *in collections of* Blepharipa pratensis *puparia, 1974*

Collection site	Number of gypsy moth pupae	Number of *B. pratensis* puparia	*B. compsilurae*	
			Number	Percent
Woodstock, Conn.	981	149	36	24
Delaware Co., N.Y.	2,285	419	75	18
New Lisbon, N.J.	4,750	874	9	1
Centre Co., Pa. (valley)	2,465	975	78	8
Centre Co., Pa. (ridge)	2,090	1,047	314	30
Total	12,571	3,464	512	15

preferred by *Brachymeria intermedia* (Minot and Leonard 1976*b*).

Other hyperparasites have been discussed rather briefly in a number of publications listing mortality agents of tachinids that attack the gypsy moth (Howard and Fiske 1911, Dalton 1976), but statistics on percent parasitism by these are usually not given.

In fall 1977, a eulophid hyperparasite,*Mellitobia* sp., was found attacking *B. pratensis* puparia stored in a field laboratory in Branford, Conn. This minute hyperparasite had escaped detection and had infested approximately 70 percent of 10,000 puparia being held for adult release the following spring. The puparia were collected from a number of locations in the Northeast, so the source of the infestation could not be specifically determined, although a subsequent collection of *B. pratensis* puparia from New Jersey also contained *Mellitobia* sp.

Mellitobia acasta (Walk.) was inadvertently brought to the United States in tachinid and sarcophagid puparia shipped from Europe to the Melrose, Mass. gypsy moth laboratory in 1906. It proceeded to infest and destroy most of the puparia being held in the laboratory, including *Blepharipa* puparia (Howard and Fiske 1911). No other references have been found that indicate recovery of *Mellitobia* from puparia of tachinids that attack the gypsy moth, so its presence in natural environments would appear to be incidental. However, this, too, is confounded by the method of collecting; that is, *B. pratensis* is usually collected as a maggot in the gypsy moth, so it is not likely that *Mellitobia* sp., which attacks the puparium, would be recovered.

Predation

B. pratensis pupae (in puparia) lie in the duff from August to May and thus are exposed to predation for a longer period of time than any of the other life stages. The fact that the puparium is out of sight in the litter probably accounts for the void of information on predation.

The first encounter with predators was in October 1974, when it was found that 255 of 300 puparia (85 percent) that had been placed in sand on the floor of a field cage in Branford, Conn., had been dug up and

the pupae inside removed, presumably by a predator. The remains of the puparia were found on top of the sand. The remaining 45 puparia were sifted from the sand and examined; all were either dried up or contained fungus. Twenty-four hours after the predation was discovered, two white-footed mice, *Peromyscus leucopus* Rafinesque, were captured in Sherman live traps placed near the former cache of puparia. Subsequent laboratory and field tests showed that the mouse and a shrew, *Blarina* sp., selectively eat *B. pratensis* puparia over gypsy moth pupae (section 6.2). Blumenthal (1975) reported predation of *B. pratensis* puparia in the litter and presented evidence that suggested raccoons were responsible.

Other Factors

The health and size of a parasite's host are known to affect the size and viability of the parasite. In the laboratory, Shapiro (1956) showed that the weight and viability of *B. pratensis* (=*Sturmia scutellata*) were directly affected by the kind of foliage its primary host, the gypsy moth, fed on. In 1973, the possibility of using the weight of the puparium as a means of predicting the survival of the pupa was assessed. Two hundred puparia collected in six different locations in Connecticut were weighed 6 to 10 days after pupariation and separated into three weight classes. These were placed, by weight class, in separate ground cages containing dirt and litter and then sealed with hardware cloth to protect against predation.

Adult emergence the following spring, by weight class, was 20–91 mg (N=88), 47 percent; 92–160 mg (N=72), 47 percent; and 61–240 mg (N=40), 50 percent. The weight of puparia as measured here does not appear to be a means of predicting survival of the *B. pratensis* pupa, or a means of determining host quality.

Field Populations

The purpose of the field studies of *B. pratensis* was to bring together all the information that had been accumulated on the parasite's life history and behavior and on sampling methods and to interpret or at least describe the fly and all related phenomena during a generation from generation to generation. Of partic-

ular interest was the interpretation of apparent percent parasitism. Almost universally, this measurement of parasitism has been based on the collection of a portion of a gypsy moth population. The stage of interest is then autopsied for the presence of parasites or reared until a parasite emerges or the insect is obviously not parasitized. For *B. pratensis* it is not known whether apparent percent parasitism means the same from one year to another. To date no use has been found for this measurement.

Ideally, the study should have been carried through one population cycle of the gypsy moth. However, program time constraints limited the study to 2 years. To partially compensate for the truncation, the study was conducted in one 0.5-ha plot in three different areas, with the gypsy moth at a different point in its population cycle in each area.

The first study area was in Eastford, Conn., on an east-facing slope of less than 5 percent. The overstory consisted of red oak (*Quercus rubra*), black oak (*Q. velutina* Lam.), black birch (*Betula lenta*), red maple (*Acer rubrum*), white oak (*Q. alba* L.), shagbark hickory (*Carya ovata* (Mill.)) K. Koch, and pignut hickory (*C. glabra* (Mill.) Sweet), in order of frequency. These ranged from 89 to 516 mm in diameter at breast height (d.b.h.). Height ranged from 7.6 to 15.2 m. This area had been 50 to 75 percent defoliated in 1973. Since then there has been a slow decline; defoliation was 20 percent in 1976 and 10 percent in 1977.

The second study area was in New Jersey in the Lebanon State Forest on the edge of the pine barrens. The overstory species on this essentially flat area were scarlet oak (*Q. coccinea* Muench.), black oak, chestnut oak (*Q. pinus* L.), and white oak. Diameters of these ranged from 51 to 356 mm, and height ranged from 9 to 12 m. Maximum defoliation, which was 50 percent in 1974, was mostly due to cankerworm. Defoliation was 30 percent in 1976 and 20 percent in 1977. Three additional study plots were put in this area in 1977.

The third study area was in Tiadaghton State Forest, Lycoming County, Pa., located on a north slope of 5 to 12 percent. The overstory consisted primarily of chestnut oak, scarlet oak, and red maple. There were a scattering of red, white and black oak

and sugar maple (*Acer saccharum* Marsh.). Diameters ranged up to 356 mm, and height ranged from 10.6 to 13.7 m. The area was 100 percent defoliated in each of the 2 study years.

In each area a grid of 50 circular 0.005-ha subplots was established. A dominant tree nearest to the center of each subplot was selected as a unit for sampling the gypsy moth population, fly eggs, maggots, leaf area, and percent defoliation.

The adult flies were counted as they emerged from hibernation. In 1976, a grid of 500 evenly spaced traps were put in each 0.5-ha area. No flies were caught. In 1977, on the basis of 1976 maggot drop counts, four traps were placed around the base of each sample tree.

The postemergent adult fly population was sampled after emergence by means of the McPhail traps. Reardon et al. (1977) have shown that this trap baited with Torula yeast caught more *B. pratensis* than either sticky traps or Malaise traps. Fifteen McPhail traps were placed in each study area; three traps were hung on each of five rope lines. On each line one trap was approximately 0.3 m above the ground, the second trap about halfway between the ground and the crown, and the third at midcrown. These were examined twice a week.

To estimate the gypsy moth population, four 171×171 mm plywood flaps were attached to each sample tree at approximately 1.2 m above the ground at cardinal points. Twice each week the larvae under these flaps were counted, the instar noted, and the number with *Parasetigena silvestris* eggs attached recorded.

Twenty-five of these trees were climbed each week, and 100 leaves were collected from the upper crown. The number of fly eggs on each leaf was counted, and the area of each leaf measured, and an estimate was made of the amount of each leaf eaten.

To determine the number of fly maggots present each week, 200 gypsy moth larvae were collected in each area. This collection reflected the age distribution of the gypsy moth population as determined by the larval census. Of these larvae, 100 were dissected and 100 were reared. To determine how many maggots survived to leave the host and pupate, five $0.25m^2$ drop traps were placed in each subplot. One

was placed around the bole of the tree and four others were placed on the ground midway between bole and crown edge. Maggots and puparia were collected twice a day. Table 6.1-23 is a summary of the data collected in 1977.

The 200 ground traps used in each area were too few to adequately sample the areas with low populations. More needs to be known about the distribution of overwintering flies in the ground before adequate samples can be made. The number of flies caught provided only the roughest estimate of the relative abundance of flies among the areas. The results were consistent, however, with the more accurate results from the McPhail traps.

Because the McPhail trap is a baited trap and there is no idea why flies are attracted to it or what proportion of the population may be attracted at any particular time, the catches can only be used to measure relative numbers. It is assumed, for the present, that the flies respond to the traps in about the same way in all the study areas. It is of interest to note that significantly more males were caught in the traps than females.

On the basis of the McPhail trap catches, four significantly different population levels occurred among the study areas: The number of flies caught in Connecticut 015 = New Jersey 105 < New Jersey 205 = New Jersey 305 < New Jersey 005 < Pennsylvania 081. This ranking on the basis of McPhail trap catches is consistent with the ranking of the study areas on the basis of fly-egg density. McPhail trap catches can be used to predict the average number of fly eggs per square meter of foliage in the study areas. The equation $Y = 2.0103 + 0.0135X$, where Y = the average number of fly eggs per square meter of leaf area and X = the total number of flies caught in 15 McPhail traps during a season, describes the relationship. The r^2 is 0.838.

Egg density is also influenced by host population density and tree species. Predictions should improve

Table 6.1-23.—*Summary of 1977* B. pratensis *population data*

Insect and stage sampled	Sampling method	Study area					
		Conn. 015	N.J. 005	N.J. 105	N.J. 205	N.J. 305	Pa. 081
B. pratensis adult	Ground trap[1]	0	3	0	0	1	139
	McPhail trap[1]	1	25	0	16	14	1,096
B. pratensis egg	Counts on leaves[2]	.29	6.13	.47	2.35	1.63	16.78
B. pratensis larva (maggot)	Drop trap[3]	0	84	2	5	4	13
	Rearing[4]	2.24	6.71	3.09	2.64	2.36	3.29
	Dissection[5]	.52	1.69	1.35	1.45	.44	16.23
L. dispar larva	Counts under bark flaps[6]	4,745	19,129	1,220	4,562	5,221	11,972

[1] Each entry is the seasonal total number of flies caught.

[2] Each entry is the seasonal average number of eggs per square meter of foliage, obtained by dividing the total number of eggs observed by the total leaf area. Total area of leaves collected in the Conn. 015 area was 57.49 m²; for N.J. 005, 80.26 m²; for N.J. 105, 76.75 m²; for N.J. 205, 76.60 m²; for N.J. 305, 80.74 m²; and for Pa. 081, 16.49 m².

[3] Each entry is the seasonal total number of maggots caught.

[4] Each entry is the apparent percent parasitism by *B. pratensis* of about 575 gypsy moth larvae.

[5] Each entry is the apparent parasitism by *B. pratensis* of about 600 gypsy moth larvae.

[6] Each entry is the seasonal total of gypsy moth larvae counted under 200 flaps.

when these factors are included in a predictive equation.

At present egg density is believed to be the best indicator of *B. pratensis* activity. What index of density (average number per square meter, highest weekly number per square meter, etc.) will be the most useful for the prediction of subsequent infection rate among gypsy moth larvae is not known. The data presented in table 6.1–24 do not generate optimism in this regard because, despite the statistically significant differences in average egg density among the study areas, there was no difference among the plots in "apparent percent parasitism" as measured by rearing collected insects. When the dissection method was used, the Pennsylvania area had a significantly higher percent parasitism than the other areas. It was also significantly higher than percent parasitism for Pennsylvania measured by the rearing method. Another inconsistency is that in some instances (Connecticut 015 and New Jersey 005) the dissection results were lower than the rearing results and in others (Pennsylvania 081) they were higher.

Two examples will illustrate why apparent percent parasitism may give a poor indication of what *B. pratensis* is doing in an area and why it has not been useful for prediction purposes. There was a significant difference in the density of fly eggs between the New Jersey 005 area and the Pennsylvania area (table

6.1–23) and no reason to believe that a larva in one area ate substantially more or less leaf area than in the other area. The expectation, therefore, was that percent parasitism would be higher in the Pennsylvania area, but it was not.

Table 6.1–24 summarizes the weekly percent parasitism in the Pennsylvania area as estimated by rearing and dissection. Peak percent parasitism is often reported in biological control surveys and in the Pennsylvania area this occurred in the week ending June 15, 1977. On the basis of dissection, however, this was a gross underestimate of the infection rate, which continued to increase in the weeks that followed. This was not evident in the rearing, because 98 percent of the larvae of the June 29 collection died prematurely of virus or of unknown causes. Although increased incidence of virus infection has not been linked with *pratensis* infection, unknown deaths, particularly among superparasitized larvae, are significantly higher among *pratensis*-infected larvae than among noninfected larvae. The results of the dissecting of larvae of the June 29 collection are shown graphically in figure 6.1–10. Note that over 40 percent of the larvae were superparasitized.

In the second example (table 6.1–25) taken from the New Jersey 005 area, there is a similar underestimation of *pratensis* infection as a result of the death of *pratensis*-infected larvae to unknown causes. At the

Table 6.1–24.—*Estimates of percent parasitism in Pennsylvania plot in 1977 by rearing and dissection of host larvae*

Date of collection	Rearing			Dissection	
	Number of larvae	Percent maggot emergence	Percent larval deaths without maggot emergence	Number of larvae	Percent with maggots
May 25	47	0.0	19.15	100	0.0
June 1	76	1.32	42.11	102	0.0
June 8	99	2.02	23.23	96	2.08
June 15	91	9.89	23.08	99	9.09
June 22	100	4.00	16.00	94	37.23
June 29	96	1.04	11.00	85	60.00
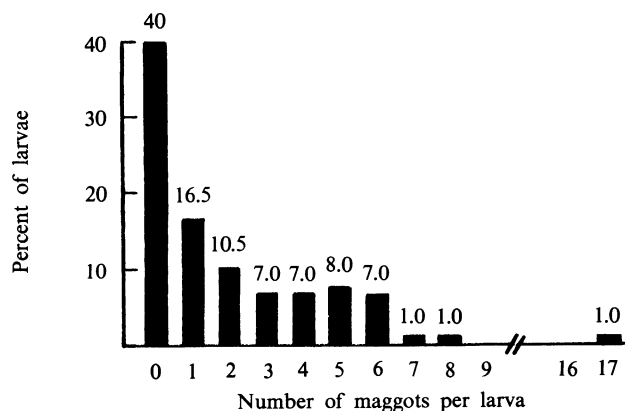
Figure 6.1–10.—*The frequency of* B. pratensis *maggots in gypsy moth larvae collected June 29, 1977, in Pennsylvania study area.*

egg density in this area, few cases of superparasitism occurred. However, unlike Pennsylvania, where there were few *P. silvestris* infections (less than 2 percent), infection by *P. silvestris* was high. Multiparasitism like superparasitism increases the incidence of unknown larval deaths. This results in underestimation of percent parasitism of both *B. pratensis* and *P. silvestris*. In addition, in those instances where multiparasitism occurs, *pratensis* survival may be reduced as much as 60 percent. Percent parasitism based on rearing would thus understate the level of *pratensis* activity in this area. These data illustrate another way in which percent parasitism, based on rearing, can lead to an incorrect conclusion. *B. pratensis* infection in 1977 appears to have been less than half of what it was in 1976. Egg density, however, did not differ in the 2 years, so the percent of larvae infected in the 2 years should have been the same, but at the same time, the incidence of *P. silvestris* increased by at least 50 percent and probably accounts for the apparent drop in apparent percent parasitism by *B. pratensis*.

Estimates of *pratensis* infection based on dissection of the host are also conservative, for the simple reason that 0.1- to 4.0-mg maggots are easily overlooked in a 1- to 2-g larva. The premature larval deaths that result from *pratensis* infection have at least two consequences for estimation of percent parasitism. First, in natural populations, infected larvae die and drop out of the population at a faster rate than noninfected larvae in the population, and this results in a certain enrichment of noninfected larvae in the population. Second, when these premature deaths take place in the course of rearing, as here, deaths are not attributed to the parasite, and estimates of percent parasitism, as measured by the rearing method, will always be conservative. They will be proportionately more so for higher fly populations because superparasitism increases with increased egg density.

What these field studies show is that the effectiveness of *B. pratensis* as a factor contributing to gypsy moth mortality is understated by current measures of apparent percent mortality. They demonstrate, too, that two areas with apparently equal levels of parasitism may arrive at those levels by quite different

routes, which may lead to quite different levels in the future.

Laboratory Culture

Source of Laboratory Colony

A technique for rearing *B. pratensis* in the laboratory has been developed (ODell and Godwin 1978). The initial laboratory stock was colonized from maggots that were trapped as they emerged from gypsy moth pupae collected in Centre County, Pa., Delaware County, N.Y., and New Lisbon, N.J.

Handling Pupae

The colonizing maggots were held in plastic petri dishes for pupariation and X-rayed 8 days later to determine pupation (ODell et al. 1974); only pupae were kept for overwintering. These were placed outdoors in dirt, sand, peat moss, and vermiculite. Survival as measured by adult emergence in these overwintering materials was, respectively, 79 percent ($N=33$), 78 percent ($N=56$), 67 percent ($N=58$), and 40 percent ($N=64$). Most of the puparia in the vermiculite were cracked and moldy. Poor drainage and icing associated with the vermiculite probably contributed to mortality.

Although this initial test of materials for overwintering *B. pratensis* pupae appeared successful, subsequent recovery of *Serratia marcescens* and other potential pathogens from dead adults and host larvae indicated a need for an aseptic rearing technique and thus a diapause-hibernation site that could be more easily controlled. At present, puparia are placed in sterile plastic petri dishes and held in water boxes at 0° to 2° C in a refrigerator. This method does not significantly change survival in comparison with holding puparia outside in sand, but by keeping puparia under refrigeration, incubation and adult emergence can be delayed up to 3 months without significantly reducing pupal survival.

King et al. (1975) reduced the incidence of infection by the bacterium *Serratia marcescens* in laboratory colonies of the tachinid *Lixophaga diatraeae* by soaking the puparium in 1.0 percent sodium hypochlorite

Table 6.1-25.—*Estimates of apparent percent parasitism by* B. pratensis *and* P. silvestris, *by rearing and dissection of host larvae from the New Jersey 005 study area, 1976 and 1977*

Insect and stage sampled	Sampling method	Year	
		1976	1977
B. pratensis egg	Counts on leaves	5.97	6.13
B. pratensis larva	Drop trap[1]	171	84
	Rearing[2]	14.9	6.7
	Dissection[3]	9.6	1.6
P. silvestris larva	Drop trap[1]	455	782
	Rearing[2]	12.5	18.33
	Dissection[3]	9.7	11.69
L. dispar larva	Counts under bark flaps[4]	25,704	19,129

[1]Each entry is the seasonal total number of maggots caught.
[2]Each entry is the apparent percent parasitism by *B. pratensis* or *P. silvestris* of about 575 gypsy moth larvae.
[3]Each entry is the apparent percent parasitism by *B. pratensis* or *P. silvestris* of about 600 gypsy moth larvae.

[4]Each entry is the seasonal total of gypsy moth larvae counted under 200 flaps.

(NaOCl) for 8 minutes. A modification of this technique was investigated as a general procedure for reducing pathogen infection in the colony. Two-week-old *B. pratensis* puparia were soaked in 1.0 percent NaOCl+0.1 percent wetting agent for 3 minutes and then rinsed twice with sterile water; a control group was treated similarly but without NaOCl. No bacterial colonies were isolated on culture plates swabbed with rinse water from the NaOCl treatment or the controls. Pupal survival was not enhanced; adults emerged from 62 percent of the control puparia ($N = 140$) compared with 52 percent emergence from the NaOCl-treated puparia ($N = 159$). The longevity and mortality of these adults were not recorded.

Attempts to shorten the normal 10-month diapause-hibernation period between pupation and adult emergence were generally unsuccessful; when puparia held outside or under refrigeration were removed from these conditions at 6, 7, and 8 months after pupation and incubated at 25° C, emergence was less than 2 percent ($N = 120$).

Handling Adults

For adult emergence, puparia were placed in open petri dishes in plastic bag cages and incubated at 23° C at a 16:8 light/dark cycle. Damp paper towels or wet cotton were placed in the cage to maintain humidity at 75 to 100 percent RH. Under these conditions pupae that had been held outside in ground cages or in water boxes at 0° to 2° C required approximately 15 days of incubation before adults began to emerge. The open container allowed callow adults to crawl to a place where the wings could unfurl and dry.

As in the field, males generally emerged first, with peak male emergence occurring about 5 days before peak female emergence. Male flies were usually maintained with the remaining unemerged puparia because mating often occurred soon after female emergence. Mating was enhanced by holding cages in indirect sunlight or by disturbing the cages.

Adult *B. pratensis* were provided water, carbohydrates (granulated sugar), and protein (honey). Handling of cages and adults was best done in the morning because the flies were relatively inactive at that time. Also, since adult *B. pratensis* are positively phototactic, light could be used to change their position in a cage or to cause flight from one cage to another.

The longevity of flies held at fluctuating temperatures of 15° to 26° C and a relative humidity of

between 50 and 90 percent averaged 15 days for males and 21 days for females that laid eggs; females that did not lay eggs lived an average of only 9 days.

The preoviposition mortality of female flies ranged from 30 to 70 percent and was generally unpredictable; however, usually if one fly died, several others in the same cage also died. Microbiological analysis of the dead flies revealed that several "potential pathogens" may have effected mortality. To reduce fly mortality, naladixic acid, a chemotherapeutic agent (King et al. 1975) was incorporated into the fly water at a rate of 100 mg per liter. The incorporation of naladixic acid did not effectively reduce adult mortality, and no further testing was carried out. The high incidence of preoviposition adult mortality is a major obstacle in the present rearing technique and will need to be overcome before a mass-rearing program is considered.

Egg Handling

In the laboratory, oviposition began 10 to 14 days after female emergence. Eggs were laid one at a time on the cage or on leaves placed in the cage. Eggs were removed from the oviposition surface with a moistened sterile insect pin and placed directly on top of the host diet; eggs pushed into the diet died within 24 hrs. Care should be taken to avoid use of host diet additives such as methy p-hydroxybenzoate, which increased the mortality in eggs that had been placed on the surface of the diet. Eggs that were not used were left on the plastic bag and stored at 0° to 2° C, 40 percent RH, for up to 4 weeks; eggs laid on leaves had to be removed to avoid mortality due to molding and leaf deterioration.

The transfer of eggs, one at a time, to the host diet is the most inefficient and costly part of the rearing technique. A leaf brush machine has been used to remove the eggs from plastic bags and leaves without affecting egg hatch or maggot establishment; however, approximately 25 percent of the eggs are lost in the process. This concept of egg removal is feasible, but before it is practical the equipment will require engineering design to prevent egg loss.

Handling Hosts

Gypsy moth larvae were reared to the fifth instar according to the technique of ODell and Rollinson (1967). Fifth- and sixth-instar larvae were reared individually in 15×100 mm plastic petri dishes or in groups of 10 in 497.65-g paper cups, depending on the research or production requirements. To ensure infection of individually reared hosts, two B. pratensis eggs were transferred to a small chunk of diet placed in a petri dish containing a fifth- or sixth-instar larva. A relative humidity of approximately 100 percent should be maintained to prevent the diet from drying out. Using this technique, infection by B. pratensis was usually achieved in 24 hours. In group rearing, feeding was achieved by placing 28-g cups filled with diet in each 497.65-g container; B. pratensis eggs were placed on diet near the edge of the cup because this is usually the section of diet consumed first. Feeding fifth- or sixth-stage larvae, 10 larvae per cup, at the rate of two eggs per larva (20 eggs per cup), is the most efficient production technique; maggot recovery (percent parasitism) ranged from 40 to 60 percent. Although the ratio of parasite eggs fed to puparia recovered was reduced by 25 percent as compared to feeding and rearing larvae individually in petri dishes, the man-hours required to rear 100 parasites were reduced by approximately 60 percent, and material costs were reduced by 90 percent.

Rearing parasitized hosts in groups in 497.65-g containers increased the weight of the female gypsy moth pupa by approximately 0.2 g and the weight of the parasite puparium by 0.05 g. Although the effect of this weight increase has not been demonstrated, it is believed that the additional weight will enhance adult fly survival, fecundity, and flight potential.

To inhibit proliferation and infection of hosts by Serratia marcescens or other potential pathogens, the antibiotics streptomycin sulfate and mycifradin sulfate and a chemotherapeutic chemical, naladixic acid, were tested individually as additives to the host diet.

The inclusion of naladixic acid in the diet (250 mg per liter) did not significantly reduce mortality and

extended the development period of the gypsy moth by approximately 4 days. On the other hand, both streptomycin sulfate (100 mg per liter) and mycifradin sulfate (75 mg per liter) inhibited the pathogenicity of *S. marcescens* without affecting the developmental rate of the host or the survival and size (puparium weight) of *B. pratensis*.

Maggots emerge from the host pupae 7 to 8 days after host pupation; some may emerge from dead larvae. Maggots were either left in the containers or transferred to plastic petri dishes for pupariation and pupal development.

During the development period, a relative humidity of around 60 percent enhances survival. Excessive moisture promotes fungal growth and appears to contribute to pupal mortality. Pupae (in puparia) were held for 6 weeks at 23° to 25° C, RH 60 percent, at a 16:8 light/dark cycle. After this they were placed in water boxes at 0° to 2° C for completion of the diapause-hibernation period.

Pupation of laboratory-reared maggots held in petri dishes for pupariation and larval-pupal apolysis ranged from 84 to 94 percent. Pupation of maggots recovered from field-collected hosts ranged from 45 to 70 percent. There are indications that the species composition of microorganisms in hosts (see Bionomics) indirectly affects parasite survival during this development period.

Summary

The past 5 years of research on *Blepharipa pratensis* have resulted in development of methods for sampling the various life stages of the fly; identification of a number of mortality-causing agents; determination of the impact of these agents on fly survival; identification of a number of cues that female flies use to determine where and when to lay their eggs; and development of a system for rearing the fly in the laboratory. It is believed that there is sufficient information about these things to attempt to manage the fly as a biological control agent.

The control tactic foreseen is the augmentation of a natural fly population in an increasing gypsy moth population. Sisojević (1975) has shown that a *B. pratensis* population reaches its maximum 1 year after the gypsy moth population reaches its maximum. It is believed that the interjection of a number of laboratory-reared flies into a natural fly population will enable that population to reach its peak sooner than it might otherwise and, as a result, have its maximum impact on the host population before the host population reaches its peak. No one knows what the actual impact will be but it is hoped that the result will be a reduction in the magnitude of the host population peak, with the peak occurring at an earlier time.

Because of the uncertainties about the nature of the impact of the parasite population on the host population, and indeed, the measurement of that impact, initial emphasis will be on the management of the parasite. Although as a practical matter of biological control it is the adult that has to be managed as an egg-delivery system, it is the number of eggs per unit of leaf area that determines the number of gypsy moth larvae infected. (In some circumstances, the use of the female may not be the best egg-delivery system. Some preliminary research indicates that eggs can be removed from the females and suspended in water, the egg suspension can be applied to the leaf surface, and gypsy moth larvae infected when the leaf is eaten. The advantages of such a delivery system are immediately apparent.) The number of eggs laid in an area can be estimated, so the initial attempt at management would be to show that the number of eggs available to the host can be increased by the release of flies.

At the moment, the number of female flies needed in an area to produce a particular egg density is not known. In areas where egg density is low, initially as many flies as can be reared and collected will be released. This will increase the likelihood that the limited number of flies released will add measurably to egg density in the areas. It has been shown that egg density is correlated to gypsy moth larval density, so on the basis of a larval census it will be possible to select appropriate areas for release.

Even if it were known how many females were responsible for a particular egg density, the problem of accounting for dispersal of the released flies still

remains. In none of the releases of any parasite to control the gypsy moth has anyone known how many of the released parasites accounted for observed differences in parasitization or how many parasites dispersed out of the study area. It has been found, however, that female flies whose eggs have matured can be conditioned to lay their eggs immediately when they encounter appropriate egg-laying cues. Therefore, the female flies will be conditioned before they are released, in order to reduce dispersal and thereby increase the number of eggs laid. This is a unique condition for a gypsy moth parasite release.

The objectives of the *B. pratensis* study have been accomplished, and the time allowed through program extension has provided the unexpected opportunity to challenge the results. It is hoped that this approach to gypsy moth parasite research sets a precedent for future investigations of other gypsy moth parasites, established or exotic.

Brachymeria intermedia (*Nees*)
(*Hymenoptera: Chalcididae*)
David E. Leonard

Brachymeria intermedia (Nees) (Hymenoptera: Chalcididae) is a solitary endoparasite of lepidopteran pupae, most notably the gypsy moth. The wasp is native to southern Europe and North Africa, where it is considered one of the primary parasites of the gypsy moth (Dowden 1935).

B. intermedia was one of the parasites imported in the parasite introduction program initiated by the Federal Bureau of Entomology and the Commonwealth of Massachusetts. Releases from France and Italy were made in 1908 and 1909 and from Sicily in 1911 and 1924-27 (Dowden 1935). These efforts to establish the parasite were considered unsuccessful (Howard and Fiske 1911, Burgess and Crossman 1929, Dowden 1935). Specimens from Spain were released in Connecticut in 1963 (Hoy 1976).

The first recovery of *B. intermedia* was in 1942 from a pupae of a leaf roller (Tortricidae) (Burks 1960*a*). In 1965, one adult was recovered on Long Island in an area where *B. intermedia* was not previously released. In 1966, the parasite was recovered in Connecticut

from gypsy moth pupae (Leonard 1966) and in New Jersey (Metterhouse 1978). Although the 1963 release may have resulted in local establishement, the wide distribution and numbers of *B. intermedia* recovered in Connecticut probably resulted from the early releases. Apparently, the insect remained at low population densities, allowing for the selection of a genotype favorable for the environmental conditions in New England. The high numbers of *B. intermedia* in Connecticut were coincidental to an outbreak of a complex of oak leaf rollers, which the parasite utilized as alternate hosts (Leonard 1967*a*).

The positive indication of its establishment and the concerns to seek alternatives to pesticides for gypsy moth control stimulated interest in *B. intermedia* as a biotic control agent for the gypsy moth. The wasp is easily cultured in the laboratory, mostly on pupae of the greater wax moth. *Galleria mellonella* (L.). The parasite has been reared in several laboratories, and field releases have supplemented the natural spread. *B. intermedia* is now commonly found throughout the current range of the gypsy moth. Only adult female *B. intermedia* overwinter successfully.

Hosts

B. intermedia is polyphagous. Dowden (1935) lists 15 species of lepidopterans attacked in its native range and found it to parasitize 10 species of lepidopteran pupae, five species of tachinid puparia, and one hymenopteran pupa in the laboratory. It has not been reported to parasitize tachinids or hymenopterans in nature. The closely related native species, *B. compsilurae* (Cwfd.), however, is a common hyperparasite of tachinid puparia, including species that attack the gypsy moth. In the United States, *B. intermedia* has been recovered from several species of leaf rollers in addition to gypsy moth. Also, in 1977, one specimen was recovered from spruce budworm (*Choristoneura fumiferana* (Clemens)) in northern New Hampshire.

Description

Burk (1960*b*) redescribed *B. intermedia* and provided synonomies and a key to the species of

Brachymeria. The following descriptions are from Dowden (1935).

Adults are large-size chalcidids, approximately 8 mm in length, with the hind femora greatly enlarged and toothed (fig. 6.1-11, *A*). The newly laid egg is about 1.0 mm long and 0.2 mm wide with rounded ends (6.1-11, *B*). The developing embryo, visible through the chorion is off white and semiopaque.

Newly hatched first-instar larvae are about 2 mm long, have 13 segments, and taper posteriorly (fig.
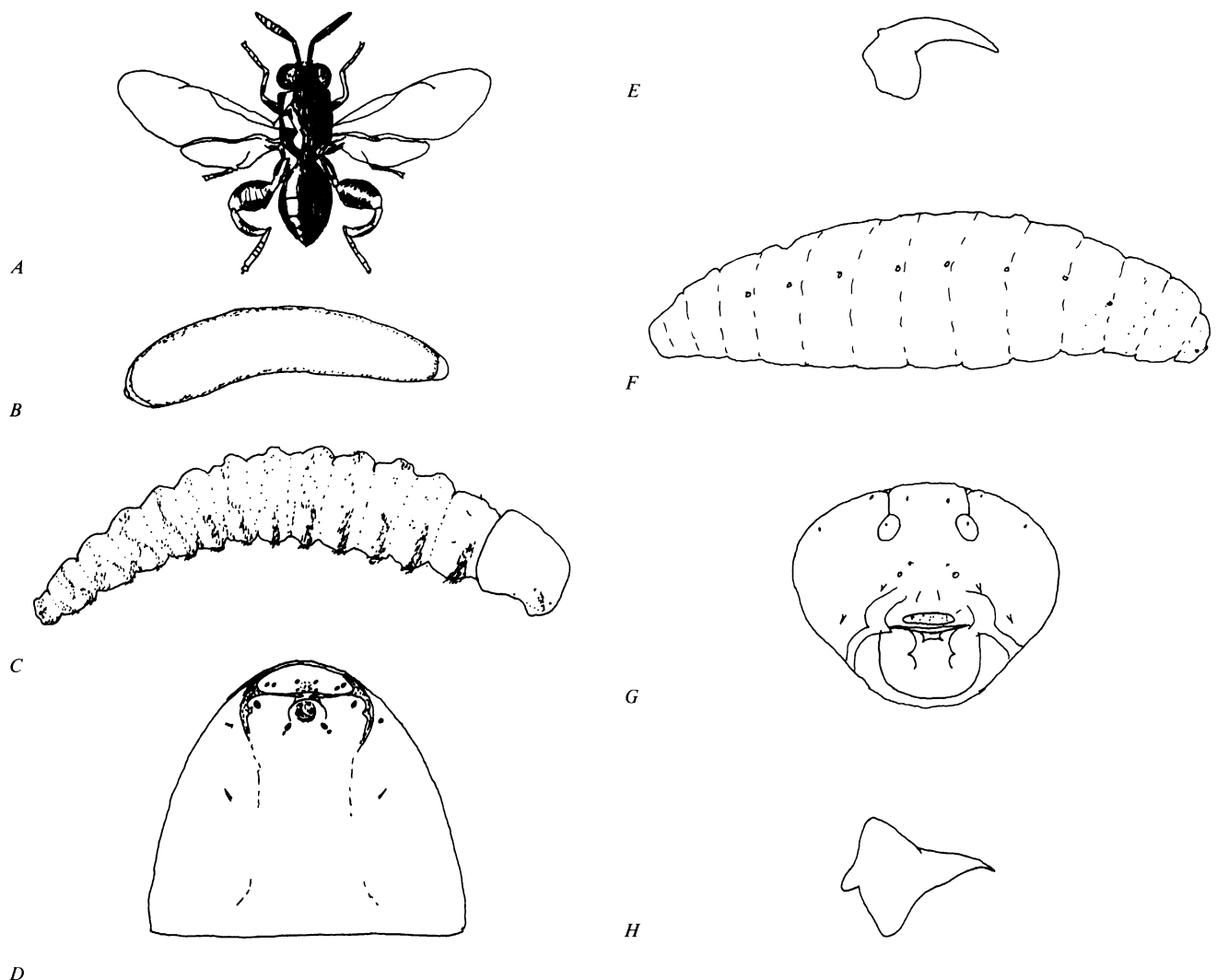
Figure 6.1-11.—Brachymeria intermedia: A, *adult;* B, *egg;* C, *first instar;* D, *head of first instar;* E, *mandible of first instar;* F, *fifth instar;* G, *head of fifth instar;* H, *mandible of fifth instar.* (*Redrawn from Dowden 1935.*)

6.1–11, C). The head is well defined (fig. 6.1–11, D) and lightly sclerotized and bears a pair of sickle-shaped, heavily sclerotized mandibles (fig. 6.1–11, E).

Second-instar larvae are similar to those in the first stage but are 2–3 mm long and more robust, and the mandibles have a broader base and are less heavily sclerotized. Third- and fourth-instar larvae are about 3–4 and 4–6 mm long, respectively, and generally resemble second-instar larvae. Fifth-instar larvae range from about 7 to 11 mm long (fig. 6.1–11, F). The head is well defined (fig. 6.1–11, G), and the mandibles are heavily sclerotized with a broad base (fig. 6.1–11, H). At the end of this stage, the white prepupa is visible through the larval cuticle.

The pupal cuticle is transparent, and adult characters can be readily distinguished. The pupal color changes from yellow to glossy black.

Biology

Dowden (1935) studied the biology of developing *B. intermedia*. One of the primary activities of first-instar larvae is the elimination of conspecific competitors. Only one larva completes development in a host. In hosts where more than one *B. intermedia* egg is laid, dead parasite larvae bearing the marks of *B. intermedia* mandibles have been recovered. First-instar larvae removed from the host attack each other when placed together.

First-instar larvae begin feeding soon after hatching. The developing larvae feed on host contents until most of the anterior portion has been consumed; the contents of smaller hosts (for example, tortricids) are entirely consumed. Each instar takes about 2 days to complete development. After a fifth-instar larva finishes feeding, it voids the meconium and becomes quiescent during the 2-day perpupal period. Pupation occurs in the host, and the pupal stage lasts about 13 days. Adults chew their way out of the host, usually near the anterior end, leaving a small, round exit hole.

Both sexes are functional, and mating can occur soon after emergence. Females apparently mate only once (Dowden 1935, Hosley 1975) but do not oviposit until 2–3 days after mating (Hosley 1975).

B. intermedia has a haploid-diploid sex determining mechanism, with males haploid. Unmated females will oviposit haploid eggs, which produce male progeny (Dowden 1935). In smaller hosts, such as spruce budworm, mated females can determine the sex of their offspring by either fertilizing eggs or laying haploid eggs, according to the size of their hosts. With this host species, a skewed sex ratio was found, with most female parasites emerging from the larger (female or large male) pupae. (Leonard 1975).

Unmated females produce fewer offspring, and the peak reproductive age is 10-24 days, compared with 5–20 days for mated females (Hosley 1975). The rate of oviposition is about 6 eggs per day per female (Dowden 1935, Hosley 1975). When held at 21° to 25° C on a diet of honey and offered no host material, *B. intermedia* lives up to 150 days. Under refrigeration, longevity can be increased still further. One female at NJDA survived 22 months at 10° C. An individual may produce 300 progeny (Metterhouse 1978).

B. intermedia females respond to chemicals (kairomones) on gypsy moth pupae; when contact with the kairomone(s) is made, walking slows, and the antennae are brought to a vertical position and vibrated rapidly up and down (Leonard et al. 1975). The gypsy moth kairomones, not yet chemically identified, are in the C–33 to C–35 (paraffin) range. Kairomones have been found on pupae of two additional species, greater wax moth and spruce budworm (Tucker and Leonard 1977). Because the parasite is polyphagous, kairomones will likely be found on other hosts as well.

In host-preference tests using gypsy moth, greater wax moth, and spruce budworm, gypsy moth was preferred (Minot and Leonard 1976a). Tucker and Leonard (1977) examined the role of kairomones in *B. intermedia* host recognition and host acceptance behavior. Females responded most strongly to filter paper impregnated with kairomones from gypsy moth, with lesser but about equal response obtained with greater wax moth and spruce budworm kairomones. The response to gypsy moth kairomone was greatest at midday. Females showed no response to greater wax moth or spruce budworm pupae that had kairomones removed with washes of *n*-hexane. A positive response to a host resulting in oviposition

involved a sequence of behaviors, including antennal contact, mounting of the host, antennal drumming, grasping of the host with the hind legs, insertion of the ovipositor, oviposition, and departure.

Temperature greatly affects the time required for development from egg to adult. The time of development can range from 2 weeks to 2 months (Dowden 1935). Males and females require an average of 24.8 and 27.1 days at 23° C and 13.5 and 15.1 days at 28° C, respectively, with twice as many parasites emerging at 28° C (Minot and Leonard 1976a).

Host species affect the developmental rate of B. intermedia (Minot and Leonard 1976a). With gyspy moth, greater wax moth, and spruce budworm, gypsy moth was most preferred but parasite development was slower than in spruce budworm, with fastest development in greater wax moth.

Host age is an important factor in B. intermedia oviposition (Minot and Leonard 1976b). Greater wax moth pupae 0–1 day old were attacked less frequently, as were pupae over 9 days old. The lack of acceptance of young pupae was thought to be related to lower levels of kairomone of fresh pupae. In older pupae, the development of the pharate adult may deter oviposition; when oviposition occurs, mortality in the developing parasites is high.

Mating Behavior

Hosley (1975) suggested that B. intermedia mating behavior did not involve an extensive sequence of male courtship behavior. A more detailed analysis (Leonard and Ringo 1978) indicates that male courtship includes a strongly sequenced pattern involving visual, behavioral, tactile, chemical, and auditory cues.

Physical Factor Preferences

Several investigators have studied the response of B. intermedia to various physical environmental factors. Minot and Leonard (1976b) found that females prefer low humidities and temperatures between 26° and 29° C. Hosley (1975) reported a similar range for male and female, with females over 5 days old selecting the higher temperatures of the preferred range. She also reported B. intermedia to be photopositive. Younger individuals preferred upper and middle sections of a gravity gradient, regardless of light conditions. Minot and Leonard (1976b) found females to be photopositive and inactive in the dark but did not find any such response to gravity. Walking activity of B. intermedia females peaked between 1330 and 1530 hours (Minot and Leonard 1976b), and response to kairomone was initiated somewhat earlier and dropped off sharply after 1400 hours (Tucker and Leonard 1977). Barbosa and Frongillo (1978) repeated many of these experiments, with similar results, and found flight and locomotory activity increased as temperature and light intensity increased.

Laboratory studies suggested that B. intermedia adults prefer warm, sunny, and dry environmental conditions in nature. Tigner et al. (1974b) found lower incidence of B. intermedia in gypsy moth pupae collected under burlap bands. Other field studies showed that B. intermedia are most prevalent in defoliated areas (Leonard 1971), at sites where the canopy is open and along the edges of the forest (Doane 1971, Barbosa et al. 1978) and that in more shaded situations, most parasitism occurs in the tops of trees (Weseloh 1972a). Higher gypsy moth population densities would be expected in such areas (Leonard 1974, Patocka and Capek 1971, Semevsky 1971). Thus, the parasite seems well adapted to orient to physical factors that are correlated with high host densities; once reaching such areas, other cues such as kairomones are important for host finding.

Effectiveness as a Biotic Control Agent

The highest rates of parasitism for B. intermedia are reported for its native range, but like the situation in North America, parasitism appears to be correlated with high host densities (Doane 1971). The highest reported parasitism by B. intermedia in the United States was 51 percent for a site in Connecticut with open canopy and woodland margins (Doane 1971). Most other reports of parasitism are much lower, averaging less than 5 percent (for example, see Tigner et al. 1974, Leonard 1966, 1967, 1971, Smilowitz and Rhoads 1973).

Role of *B. intermedia* in Integrated Pest Management

What will be the role of *B. intermedia* in integrated pest management approaches for gypsy moth? *B. intermedia* is easily cultured and establishes quickly after release. Smilowitz and Rhoads (1973) found that within several years of release in Pennsylvania the parasite was recovered in most of the counties where releases were made. Efforts will probably continue to establish *B. intermedia* along the leading edge of the range of the gypsy moth.

The analysis of physical factor requirements and confirming evidence from the field suggest that *B. intermedia* is most prevalent where gypsy moth population densities are either high enough to cause defoliation or occur in more open areas. A parasite that is most effective at high host densities will not have much impact on reducing the host population. Since low host densities where no defoliation is occurring will not provide the preferred physical factor requirements of *B. intermedia*, little parasitism can be anticipated when gypsy moth population densities are low.

B. intermedia has two, possibly three, generations a year. The numbers of this parasite available to attack the gypsy moth will depend on the availability of alternate hosts for the previous generation.

Enhancement in rate of parasitism with the use of kairomone has not been evaluated. The kairomone(s) are not volatile, however, and would not attract *B. intermedia* from long distances. It is anticipated that *B. intermedia* would remain in an area treated with kairomone, and host searching would probably be stimulated. Field trial would require identification and synthesis of the kairomone.

Anastatus ?kashmirensis *Mathur*
William E. Wallner

Despite the fact that two gypsy moth egg parasites, *Ooencyrtus kuvanae* and *Anastatus disparis Ruschka*, are known to be established in North America (Parker 1933), other candidates were being sought for introduction through the Expanded Gypsy Moth Program and the Beneficial Insect Introduction Laboratory. *Anastatus ?kashmirensis Mathur*, a eupelmid parasite collected from the Indian gypsy moth *Lymantria obfuscata*, was considered a prime species for introduction. During 1975 specimens were received from India, and laboratory colonization was initiated because the insect is easily reared on both unembryonated and embryonated gypsy moth eggs.

A. ?kashmirensis is morphologically very similar to *A. disparis*, although the species has only been tentatively identified as *kashmirensis* and is still under taxonomic study. The two species can be separated only with great difficulty on the basis of coloration of the hind tibia of males (Gordh 1977). However, cross-mating studies indicate that they are reproductively isolated. Comparisons of biological habits (overwintering, number of generations per year, survival) of these two *Anastatus* spp. have not been done and could not be ascertained in these studies. This was because only laboratory evaluations of *A. ?kashmirensis* were conducted; field evaluation was considered too hazardous because of the minute size (1.8 to 3.0 mm) of adults. Accordingly, studies were designed to compare *A. ?kashmirensis* with the most effective gypsy moth egg parasite, *O. kuvanae* in the United States.

It was found that *A. ?kashmirensis* could not oviposit any deeper into *L. dispar* egg masses than *O. kuvanae*. The resulting parasitization rate was 27 percent for *O. kuvanae*, and 6 percent for *A. ?kashmirensis*. Hence, *A. ?kashmirensis* is capable of capitalizing only on those eggs already heavily parasitized and probably would not contribute significantly to the present parasite complex.

The role of parasite introduction in biological control programs has been a controversial topic among biocontrol workers. Some researchers believe that only the most effective parasites should be introduced, which requires preintroduction evaluation (Turnbull 1967). A major concern is creation of a competitive role among indiscriminantly released parasites, which reduces their overall effectiveness. Yet others (Huffaker et al. 1971) feel that, since there have been few instances of reduced control due to multiple introductions, predicting the outcome is impossible.

The accepted procedure in dealing with known facultative hyperparasites is considerably more complex. While *A. disparis* is known to hyperparasitize *A. melanoscelus* (Ratzeburg) in the United States, it was not released with this knowledge. Because other members of the genus *Anastatus* have hyperparasitic tendencies (Clausen 1972), *A. ?kashmirensis* hyperparasitic tendencies were considered critical. While it might be difficult to identify the most effective parasite, it should be feasible by laboratory evaluation to eliminate ineffective or detrimental species.

A series of laboratory choice studies were conducted in which adults of *A. disparis* and *A. ?kashmirensis* were offered *L. dispar* egg masses and *A. melanoscelus* cocoons. *A. ?kashmirensis* was most often associated with *A. melanoscelus* cocoons, whereas *A. disparis* preferred *L. dispar* eggs. *A. ?kashmirensis* preferred cocoons in spite of preexposure to eggs and cocoons.

In still another experiment of choice, adult *A. ?kashmirensis* and *A. disparis* were offered *A. melanoscelus* cocoons, *L. dispar* eggs less than 2 days old, and *Rogas indiscretus* Reardon mummies. Adult *A. ?kashmirensis,* while attacking *L. dispar* eggs more often also oviposited in cocoons and mummies. *A. disparis* almost exclusively attacked *L. dispar* eggs.

A. ?kashmirensis will successfully complete development in *R. indiscretus* mummies and *A. melanoscelus* cocoons. Progeny from *A. melanoscelus* were three to four times larger than those reared from gypsy moth eggs, and progeny from *R. indiscretus* were even larger. This difference was believed to stem from the larger host, which supported more growth. Interestingly, adult *A. ?kashmirensis* females reared from *A. melanoscelus* or *R. indiscretus* did not have a preference for attacking the same host species from which they were reared over gypsy moth eggs masses. This is interpreted as facultative hyperparasitic association.

The impact of *A. ?kashmirensis*, whether beneficial or detrimental, cannot be ascertained without actually releasing it. It has not been recovered from *Apanteles* spp. cocoons collected in India (R. C. Hedlund, ARS, in letters of December 30, 1977, and January 20, 1978). However, it is not known that *A. ?kashmirensis* was present in the same location at the time collections were made. Furthermore, *A. ?kashmirensis* parasitizes at least one other braconid parasite (*R. indiscretus*), which suggests that other parasites important in pest regulation also could be adversely affected.

Summary and Conclusions

It was recommended that *A. ?kashmirensis* not be released. This decision was based on the anticipated ineffective role of *A. ?kashmirensis* as a primary gypsy moth parasite, its propensity as a facultative hyperparasite, and the difficulty of separating morphologically the two *Anastatus* species. A further confounding influence was the fact that while all *A. disparis* diapaused in *L. dispar* eggs, *A. ?kashmirensis* emerged. These emerging *A. ?kashmirensis* adults would be free to attack other gypsy moth egg masses; however, *A. ?kashmirensis* prefers freshly laid eggs and would therefore attack other parasites, thus proving to be additionally deleterious.

Apanteles melanoscelus *(Ratzeburg)*
Ronald M. Weseloh

Apanteles melanoscelus (Ratzeburg) is a hymenopterous (wasp order) solitary internal larval parasite of the gypsy moth introduced from Europe to New England in 1911 and in following years. It is currently established widely in most States infested with the gypsy moth. It overwinters as a large larva within a cocoon attached to the bark of trees, under bark flaps or in any location where its host happens to be when the parasite emerges from within it. Adults emerge from cocoons and parasitize gypsy moth first- and second-instar larvae in spring. Progeny develop without diapause to adults that attack second- and third-instar larvae in June. These progeny enter diapause after spinning cocoons in late June and early July.

General Biology

Information on the basic biology of *A. melanoscelus* has been given by Crossman (1922), and the parasite has recently been studied with some intensity

with the result that a relatively large amount of information is now known about it.

In the laboratory, females mate and are able to oviposit within 2 to 3 hours after emerging as adults. They may live for 3 to 4 weeks and can lay up to at least 500 eggs in their lifetime. Eggs, which are injected directly into the body cavity of the host, hatch in about 2 days, and the larval parasites (one per host) go through three stadia, each of which can be 2 to 8 days long (in nondiapausing individuals). Third-instar larvae emerge through the host integument and spin yellowish-white cocoons. Depending on photoperiod (see Diapause and Hybridization), the third-instar larva inside the cocoon either pupates within 2 days or enters diapause for several months before pupating. The pupal stage lasts 5 to 9 days. Adults emerge by pushing off a cap at the anterior end of the cocoon.

Hyperparasitism

Because *A. melanoscelus* is present in a cocoon from July until the following May, it is especially vulnerable to attack by hymenopterous hyperparasites including species from the families Ichneumonidae, Pteromalidae, Eulophidae, Encyrtidae, Eupelmidae, and Eurytomidae. Most of these attack nonparasitic insects as well as parasites and have large host ranges. They have been known to parasitize up to 80 or 90 percent of overwintering *A. melanoscelus* cocoons (Grimble and Palm 1976, Van Sickle and Weseloh 1974).

Their attack patterns have been studied by exposing laboratory-reared cocoons of *A. melanoscelus* in various forest microhabitats for 1-week periods and then rearing out the hyperparasites (Weseloh 1978). *Eurytoma verticillata,* (F.), the most abundant hyperparasite, restricted its activities mainly to the trunks of trees. Other hyperparasites, often abundant on the trunk but not to the extent of *E. verticillata,* also attack cocoons on leaves. *E. verticillata* was instrinsically superior to *Gelis tenellus* (Say), an ichneumond hyperparasite, in laboratory tests. That is, if *G. tenellus* and *E. verticillata* both laid eggs in a single *A. melanoscelus* cocoon, *E. verticillata* was almost always the one that survived, even if *G. tenellus* had oviposited many days before.

It is reasonable to suspect that the distributions of other hyperparasites in the field are influenced by the intrinsic superiority of *E. verticillata.* To determine this, *A. melanoscelus* cocoons placed on tree trunks and leaves were observed periodically to see what species of hyperparasites oviposited where. Other hyperparasites as well as *E. verticillata* were more often observed on cocoons located on trunks than on leaves, strongly suggesting that *E. verticillata* eliminates other hyperparasites after eggs are laid and thus leading to the observed parasitization patterns.

Host/Parasite Synchronization

In addition to the depredations of hyperparasites, *A. melanoscelus* is hampered by lack of synchronization with its host (Weseloh 1976*b*). In the laboratory, *A. melanoscelus* females readily parasitized gypsy moth instars one to three, but not instar four. This appeared to be primarily because of active defensive movements and the long hairs of the large larvae. Also, at three different temperature regimes development of *A. melanoscelus* from egg to adult always took longer than the development of gypsy moth larvae from the first to fourth instar.

Because of these differences in development, adults of the overwintering generation in the field reproduced quite well, but their progeny matured after most hosts were too large to be suitable. Adult parasites reached maximal numbers in mid-June when most gypsy moths were in the fourth instar or larger. Percent of parasitism of larvae at the same site declined beginning the first of June, probably reflecting the decreasing suitability of hosts.

Diapause and Hybridization

Diapause in *A. melanoscelus* is induced by short day lengths, with late-instar larvae being most sensitive to photoperiod (Weseloh 1973). The critical photoperiod depends on the strain used. Hoy (1975*a*) found that a laboratory strain from Connecticut had a critical photoperiod of about 16 hours of light per day; for two laboratory strains from Europe (France and Yugoslavia), the critical photoperiods were each about 13 hours of light per day. Differences could

have been due to different rearing procedures, because a new strain freshly collected in France was similar to the Connecticut strain in its diapause response. Hybridization between the Connecticut strain and the two older European strains resulted in a form with intermediate diapause characteristics. These genetic differences were probably a result of inadvertent laboratory selection. Such selection could prevent the induction of diapause in the field and thus the establishment of otherwise desirable strains.

Hoy (1975*b*) also tested different strains and hybrids of *A. melanoscelus* in the laboratory for developmental rate, host attack rate, and sex ratios. Of the five strains and two hybrids tested, a field-collected Connecticut strain (the only one which had not been reared previously in the laboratory) developed fastest, produced the most progeny, and had the highest percent of female progeny. This work shows the effect that laboratory rearing can have on a parasite's genetic makeup. A release of a triple hybrid of three laboratory strains against the gypsy moth in the field was no more effective than release of a nonhybridized strain.

Mating Behavior

The mating behavior of *A. melanoscelus* involves perception of the female by the male from a distance of at least 5 mm, male wing elevation and fluttering, mounting the female, and copulation. Aspects of mating behavior have been investigated by Weseloh (1977*a*). Virgin females, no matter what their age, are receptive to males, but older males do not respond to females nearly as readily as do younger males. Females can prevent mating by moving away when contacted by males, and quiescent females have a greater chance of mating than active ones.

A hexane-soluble sex pheromone is produced in the female's reproductive system (Weseloh 1976*c*); when placed on a piece of filter paper, it releases wing-fluttering behavior in males but not copulatory attempts. However, if a dead male is glued to the filter paper and the pheromone extract is applied, the fluttering is more intense and copulatory attempts occur. The model used to represent the female does

not need to be very complete; dead males with legs, wings, or antennae missing are effective. No such response occurs when only the model is present. These experiments show that the pheromone serves to identify an object as a female and that tactile and/or visual stimuli are necessary for precise orientation and complete expression of mating behavior.

Host-Selection Behavior

The host-selection process in any parasite is hierarchical in nature: It first involves the selection of a habitat or microhabitat, then the finding of the host within that habitat, and finally the recognition and parasitizing of the host, or avoidance of it if it is already parasitized (Doutt 1964).

Little direct information is available on habitat selection by *A. melanoscelus*. Weseloh (1972*a*) exposed clear sticky panels at different heights in trees and found that *A. melanoscelus* adults were caught primarily in the canopies rather than near ground level. Tigner et al. (1974) collected gypsy moths under burlap on trees near ground level and at 8.5 m, but found little differences in percent of parasitism by *A. melanoscelus* at the two heights. Colored sticky panels were also exposed near ground level by Weseloh (1972*b*). These did not trap *A. melanoscelus* adults to any extent, but a related parasite, *A. laeviceps* Ashmead, was caught readily, most often on clear (which transmitted foliage colors) and yellow panels. This suggests that the parasite was responding to foliage, and possibly *A. melanoscelus* does so as well.

The process used by *A. melanoscelus* to actually find hosts is better known than those processes involved in habitat selection. When a female encounters strands of gypsy moth silk (normally deposited on leaves when small gypsy moth larvae feed) (Leonard 1967*b*, 1970), she intensely examines the area surrounding the silk with her antennae (Weseloh 1976*d*). The parasite can detect as few as 10 silk strands on a 1 cm² substrate, and the response is specific to gypsy moth silk. Contact with the silk apparently activates the parasite and causes it to discover nearby hosts; when the gypsy moth larvae are near or present on substrates covered with silk strands, they are attacked and parasitized more

frequently than similar larvae not associated with strands. A water-soluble active chemical, known as a kairomone, can be extracted from the dissected silk glands. Parasites will respond to it after it has been deposited and dried on thin strands, such as cotton fibers. The kairomone is stable at temperatures from at least $-18°$ to $100°$ C, is nondialyzable, and is not deactivated by treatment with ethanol (Weseloh 1977b).

The process functions as an efficient host-finding mechanism if one assumes that *A. melanoscelus* females are initially attracted to leaves but only examine them briefly unless they contact fresh gypsy moth silk (that is, silk still retaining kairomone not washed off by dew or rain). They then intensely examine the leaf and surrounding areas to find the gypsy moth larva that is probably there.

Once the host is contacted, other criteria lead to acceptance or rejection. Weseloh (1974) showed that gypsy moth larvae killed by freezing were as acceptable as living ones to *A. melanoscelus* females. Females also probed as much in host exuviae as in dead larvae. However, dead larvae from which body hairs were removed were not as acceptable as hairy dead larvae. Hairs removed from larvae were effective in eliciting examination behavior by the parasite, indicating that the hairiness of hosts is important in the recognition process.

Females responded less to gypsy moth larval exuviae soaked 24 hours in methyl chloride or hexane than to nonsoaked exuviae. This suggests the presence of a cuticular chemical (kairomone) that the parasite perceives. Leonard et al. (1975) found that hexane extracts of gypsy moth larval integuments, when deposited on filter paper and allowed to dry, would cause examination behavior in *A. melanoscelus* females when they contacted the spot, thus confirming the presence of the kairomone.

A. melanoscelus females also have the ability to discriminate between parasitized and nonparasitized hosts (Weseloh 1976a). The discriminatory ability, however, is not perfect, because caterpillars tend to be superparasitized if a female has access to only a few hosts. If more than one egg is laid in the same host at about the same time, supernumeraries are eliminated by active combat between first-instar parasites. The

discriminatory ability of *A. melanoscelus* does not seem to be affected by age differences of parasitized hosts.

Concluding Remarks

Information now exists on mortality factors, host synchronization, aspects of diapause and laboratory genetic selection, and mating and host-selection behavior of *A. melanoscelus,* making this parasite better known than the majority of entomophagous parasites (including gypsy moth parasites). As such it should be possible to use the insect more effectively through augmentative and manipulative procedures . However, many aspects of its biology still need elucidation, especially habitat selection in the field, host suitability, population biology, and genetics. When these areas are explored, additional possibilities for practical application will probably be found. Continued study of this species is likely to be worthwhile.

Parasite Augmentation
E. Michael Blumenthal, Robert A. Fusco, and Richard C. Reardon

Introduction

Manipulation techniques to initiate or increase effective biological control by established parasites are generally applied only as a last resort in an attempt to regulate pests in a forest ecosystem. In the case of gypsy moth, these techniques were applied after it was determined that previously established parasites were not regulating the pest and that over 90 percent of the newly imported natural enemies apparently were not becoming established. Manipulation has not been tested satisfactorily as these methods are complex by nature, expensive to develop, and generally difficult to apply. Also, at this time there are insufficient data on the life history and biology of specific parasites and parasite complexes.

It is apparent in biological control literature that the terminology used by various authors to describe parasite releases is often arbitrary, contradictory, or cautiously vague. Although the basic concepts were

described by DeBach and Hagen (1964), many workers have chosen to qualify the definitions by delimiting release parameters such as the size of the treatment area (Turnock and Muldrew 1971) or numbers of parasites released (National Academy of Science 1969). Others have amended or supplemented the terminology itself, resulting in a nearly inseparable array of "descriptive" concepts. For our purposes, the definitions of DeBach and Hagen (1964) will be used unless otherwise noted.

As has been pointed out in previous sections, most efforts devoted toward biological control of gypsy moth have been "classical" parasite releases—*importation and colonization.* The intent in these releases was not to manipulate but to establish exotic parasites in areas foreign to the species. Importation and colonization have been primarily trial-and-error efforts, inexpensive to apply relative to other control methods, and usually succeeding or failing without repeated releases or additional help from man. Manipulation of the environment (*conservation*) may be required, however, before an imported parasite can become established, but this is a difficult task in forest ecosystems and has not been attempted with gypsy moth parasites.

Manipulation of established parasites, termed *augmentation,* may employ either of two release techniques, *inoculation* or *inundation,* both of which are defined on the basis of expected results. Both are periodic colonizations (augmentation) requiring that the releases be repeated, as necessary, to maintain continually a pest population below an economic or social threshold. Augmentation implies that an established parasite is inherently incapable of self-perpetuating biological control, regardless of whether the deficiency is intrinsic or environmental (DeBach 1974, Huffaker et al. 1977).

Inoculative Releases

Inoculation refers to the release of parasite breeding stock whose progeny are expected to exert a regulating influence on the pest in subsequent generations. Inoculative releases have been used extensively, and successfully, for control of agricultural and horticultural pests (DeBach 1974).

Small-scale releases of this type have been attempted against the gypsy moth in Pennsylvania and New Jersey during the last decade, although no significant release effects have been noted (New Jersey Department of Agriculture 1967–76, Pennsylvania Department of Environmental Resources 1973–77). These releases have involved relatively small numbers of parasites whose effects have been evaluated within small sampling areas encompassing the release points. Parasite dispersal beyond the sampling areas apparently has hampered the effectiveness of the parasite releases.

Not until recently, however, has this technique been attempted on a scale large enough to evaluate release effects with minimal impact by parasite dispersal. The two independent studies to be discussed here were prompted by an unpublished parasite augmentation model proposed by Knipling (1972). The model suggests that release of adequate numbers of an established parasite against its preferred host stage at a specific host density would result in progressive parasite population increase and, consequently, accelerated host reduction. For the parasite described in the model, *Brachymeria intermedia* (Nees), optimum gypsy moth and parasite densities were about 74 egg masses per hectare and 247 parasites per hectare. Regardless of host density, parasitism was to approximate 50 percent in the year of release, thus nullifying the lag time usually required for parasites to reach effective levels (Huffaker et al. 1971, Knipling 1972). The Pennsylvania (Blumenthal et al. 1978) and New Jersey studies both employed two established parasites in inoculative releases, *B. intermedia* and *Compsilura concinnata* (Meigen).

Pennsylvania Studies

The Pennsylvania study locale, located about 24 km northwest of Reading, Pa., was characterized by at least 50 percent overall oak composition; a post-culmination gypsy moth population (1972 collapse); and prerelease egg-mass counts of about 74 and 334 egg masses per hectare in the *B intermedia* and *C. concinnata* study locales, respectively. Four circular study areas were established for each of the test species. The areas each encompassed about 146 ha

and were separated by at least 1.6 km. There were 33 sampling points in each *C. concinnata* area and 53 plots in each with *B. intermedia*. Two treatment and two control areas were selected at random from each set of four study areas. The effects of the inoculative releases were evaluated weekly from larval collections in the *C. concinnata* areas and from pupal monitoring (tagging and weekly observations) and collections in *B. intermedia* areas.

Over 83,650 flies (sex ratio 1:1) and 78,150 wasps (sex ratio 1:4, male to female) were released in the *C. concinnata* and *B. intermedia* treatment areas, respectively. Releases of *C. concinnata* were made on predominantly third-stage host larvae. *B. intermedia* adults were released from the time of first pupation observed to peak host pupation. All releases were made in 1976 using mated adult parasites, samples of which were field-cage tested for aggressiveness and fecundity.

Gypsy moth larval collections in 1976 and 1977 totalled 67,349 and 32,360, respectively, from *C. concinnata* areas. Evaluations in *B. intermedia* areas involved 6,480 and 3,360 monitored pupae and 3,224 and 2,288 collected pupae in 1976 and 1977, respectively.

Apparent parasitism by *C. concinnata* was significantly different between treatment (4.4 percent) and control (2.0 percent) areas in 1976, although this is not considered significant from a pest-management perspective. This difference did not persist in 1977. Extensive dispersal and unavailability of alternate hosts are proposed to explain low parasitism rates.

The release of *B. intermedia* had no apparent impact on hosts sampled by either evaluation technique in 1976 and 1977; apparent chalcidid parasitism of all collected pupae averaged about 0.2 and 0.4 percent, respectively, and of all monitored pupae, about 1.5 and 1.7 percent. *B. intermedia* parasitism did not differ between pupae observed near the ground and those in the forest canopy. It is suggested that the released parasites dispersed *en masse* beyond the periphery of the 23-km² study locale.

For both species, *C. concinnata* and *B. intermedia*, the increase in parasitism (2 percent and 0 percent,

respectively) between the treatment and the control areas was significantly less than the 50 percent projected by the Knipling model.

New Jersey Studies

The New Jersey *C. concinnata* study was conducted on a 11.5-ha release site in Ocean County, N.J. Within this site, five sampling/release stations were established, each with 10 burlap-banded host trees for larva and pupa collections. A check site, containing one sampling station with 10 burlap-banded trees, was located about 1.6 km away. Prerelease estimates of gypsy moth density were 124–247 egg masses per hectare.

Approximately 7,110 *C. concinnata* adults and puparia were released throughout the five sampling/release stations.

Host collections in the release and check sites totalled 1,147 and 688 gypsy moth larvae and pupae, respectively. One *C. concinnata* was recovered from collections in release sites and two from the check site. In the fall of 1976, egg-mass surveys indicated 44 egg masses per hectare in the release site, and 563 egg masses per hectare in the check site.

Collections in 1977 resulted in release- and check-site totals of 552 and 316 larvae and pupae, respectively. One *C. concinnata* issued from release-site collections; none was recovered from the check site. Release- and check-site egg-mass density estimates in the fall of 1977 were, respectively, 5 and 15 egg masses per hectare.

The *B. intermedia* study conducted by the New Jersey Department of Agriculture employed 27 ha of forest in Hunterdon County, N.J. Five subplots were established in this area. Within each subplot, 10 cardboard bark flaps were stapled to gypsy moth host trees to provide larval resting niches. An additional 60 cardboard bark flaps were constructed at 12 sampling stations 61 m apart along a compass line. Prerelease gypsy moth density was about 74 egg masses per hectare.

About 11,000 adult *B. intermedia* (sex ratio .54:1, male to female) were released within the five subplots. Weekly host collections in the subplots accumulated 194 pupae from which no *B. intermedia* emerged. One

B. intermedia exit hole was observed during field examinations of 161 empty pupa cases found at compass-line stations. Fall 1976 egg-mass counts indicated an average of 49 egg masses per hectare.

Host collections in 1977 produced 276 pupae, and three adult *B. intermedia* were recovered. The compass-line stations yielded 217 pupa cases, none of which appeared to have a *B. intermedia* exit hole.

The conclusions of the New Jersey study were that the objective of 50 percent parasitism of gypsy moth was not achieved by inoculative release of either *C. concinnata* or *B. intermedia*, and that the reason for the lack of parasitism by *C. concinnata* is not known. The reason for the lack of parasitism by *B. intermedia* is that this species "is effective only in high-density gypsy moth populations where a great deal of sunlight penetrates the forest canopy because of heavy defoliation."

Inundative Releases

Inundation describes parasite releases expected to produce immediate control as a direct result of parasitism by the released individuals. The parasites in this context have been referred to as biotic or biological insecticides (Stinner 1977). Popular usage implies that exotic parasites may be employed as inundative agents (Pschorn-Walcher 1977).

Inundative releases of five species of gypsy moth parasites *Anastatus disparis* Ruschka (Hymenoptera: Eupelmidae), *Apanteles melanoscelus* (Ratzeburg), *A. liparidis* (Bouché), *A. porthetriae* Muesebeck (Hymenoptera: Braconidae), and *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) have been made in several geographic locations in an attempt to suppress populations of the gypsy moth.

Anastatus disparis

This parasite was selected for release in Spain because large numbers of parasitized eggs could be easily collected and be mass reared with minimal difficulty. A total of 1.5 million parasitized gypsy moth eggs were placed in a 100 ha-area (200 eggs per paper bag per tree). *Anastatus* adults would then develop and issue at the time that the gypsy moth eggs

were laid. Following the releases, high rates of egg parasitism were recorded. Unfortunately, because of chemical treatment, the gypsy moth population the following year was sparse and the parasite population could not be surveyed (Romanyk 1965).

Apanteles melanoscelus

This insect was selected for inundative release because it is easily reared in the laboratory, lacks synchronization with the host, and is susceptible to extensive overwintering mortality by hyperparasites.

Dowden and Reardon (1967) released about 1,500 females in an isolated gypsy moth infestation in the southern Lake Champlain region in Vermont. The release plot was a 16-ha stand of large white oaks with a dense 4,940 egg masses per hectare gypsy moth population. Because of extensive mortality of over-wintering eggs, larvae were difficult to collect. There were no significant differences for percent parasitism by *A. melanoscelus* between treatment and control plots on the basis of collections of host larvae throughout the plots and of *A. melanoscelus* cocoons beneath burlap bands.

Tigner (1974*b*) studied *A. melanoscelus* populations in New York State, using burlap bands to collect gypsy moth larvae and *A. melanoscelus* cocoons. He found that *A. melanoscelus* did not respond positively to concentrations of gypsy moth larvae and that adult parasites are not likely to disperse very far from the point of release. The implications of Tigner's study suggest that a release of *A. melanoscelus* at one location would not have a uniform measurable effect upon a larger infestation but might cause an impact upon small concentrations of gypsy moth larvae.

In 1974, Vasić (1976) placed 1,200 *A. solitarius* (Ratzeburg) (probably *melanoscelus*) cocoons per treatment plot in two forested areas in Yugoslavia. At both areas, two plots measuring 10 × 10 m were marked, one as control, one as treatment. In comparison to the controls, parasitism by the species in one area increased 9.5 percent and in the other, 12.1 percent. These results are difficult to interpret because identification of the *Apanteles* species released is uncertain, thereby complicating the relationship

between the recorded parasitism percentages and the species released.

Weseloh and Anderson (1975) released *A. melanoscelus* pupae and adults on three sites (Thompson, Wolcott, and Eastford) in Connecticut. Each site comprised several plots, each 4 ha. The Eastford site supported a dense (1,570–1,800 egg masses per hectare) host population, while on the Thompson or Wolcott sites no egg masses were observed, and artificial populations were established by introducing 72,000 eggs per plot and 48,000 eggs per plot, respectively. In 1973, a total of 6,671 *A. melanoscelus* cocoons (about 2,000 females) was placed in one plot in Thompson; in 1974, 6,144 and 6,125 cocoons were placed in two plots in Wolcott; and 1,719 cocoons were placed in each of two plots in Eastford. In Thompson and Eastford, collections were made primarily around the center tree (release point), while in Wolcott both the center tree and quadrant tree areas (release points) were sampled. At least 2 to 3 weeks after release, collections of larvae mostly from beneath burlap bands indicated a greater percent parasitism (up to 40 percent in low-density areas) by *A. melanoscelus* in the release plots than in the check plots at all three sites. In later collections, no differences occurred in the Thompson or Wolcott sites. In the Eastford site, however, percent parasitism in the release plots was higher than in the checks even at the seventh weekly collection following release.

In 1974, Grimble (1975 *b*) released 4,000 female *A. melanoscelus* per plot in two plots, each plot having three laterally spaced 0.4-ha subplots, in a dense (average 10,000 egg masses per hectare) gypsy moth population in Delaware County, N.Y. There was no significant difference in percent parasitism by *A. melanoscelus* among release and control plots based on host larvae collected randomly from the ground (parasitism averaged less than 2 percent) and counts of *A. melanoscelus* cocoons beneath burlap bands.

Ticehurst and Fusco (1976) released 1,000 females per 0.5-ha plot in three eastern Pennsylvania plots in 1975, where gypsy moth population density averaged 52.5 egg masses per hectare and ranged from 25 to 3,300 egg masses per hectare. Host larvae were collected from the canopy of oak trees, and apparent

parasitism never exceeded 2.2 percent for any sample. There were no significant differences in percent parasitism by *A. melanoscelus* between treatment plots and control plot for any week or combination of weeks.

Although several of the inundative releases of *A. melanoscelus* resulted in significant increases in parasitism by *A. melanoscelus*, none had an impact on host population density based on egg-mass counts. In addition, apparent parasitism percentages for *A. melanoscelus* are difficult to interpret among studies because of collection of hosts at various strata (Tigner et al. 1974, Weseloh 1972), use of various collection techniques (Reardon 1976), and use of artificial and natural populations.

The value of inundative release of *A. melanoscelus* against the gypsy moth remains uncertain at this time, although Weseloh (1977*c*) has made two recommendations for increasing the effectiveness of *A. melanoscelus* by augmentation: Develop a strain of *A. melanoscelus* that would attack fourth-instar gypsy moth larvae as well as the smaller larvae (the new strain might be developed by deliberate selection or perhaps found through foreign exploration); and ensure survival of more of the overwintering generation by controlling the hyperparasites (the effect of hyperparasitism may be indirectly diminished by rearing and releasing the parasite in areas where gypsy moths are expected to increase). The effectiveness of this procedure will depend on, among other things, the degree to which parasites remain in the areas in which they are released. It may be possible to improve retention by depositing the silk kairomone on tree leaves so the parasites will be induced to examine intensely the area for hosts and not disperse, which would result in greater parasitism of gypsy moths where most needed.

Hoy (1975*b*) released a triple hybrid FYC (French × Yugoslavian × Connecticut strains) in 1974 near Wolcott, Conn., in an effort to obtain a more effective parasite for inundative release through increased genetic diversity. Since egg masses were not observed in three release and check plots, artificial populations were established by placing 48,000 eggs in each plot. Totals of 6,186, 6,109, and 6,086 FYC-hybrid cocoons

were placed in the respective plots. Results of weekly collections of gypsy moth larvae made at the center and around quadrant trees showed that the percent parasitism by *A. melanoscelus* was significantly higher in release plots than in check plots.

Apanteles liparidis and Apanteles porthetriae

These two exotic species were selected for inundative release because both are easily reared in the laboratory and *A. liparidis* has a tremendous reproductive capacity. The New Jersey Department of Agriculture (NJDA) in 1975 released approximately 2,500 female *A. liparidis* per week for 10 and 12 weeks at each of two sites, each site measuring about 146 ha. The Earle site had a dense 801 egg masses per hectare host population, whereas the Lakeview Drive site had a sparse, average 185 masses per hectare population. No recoveries of *A. liparidis* (total of 23,500 females released) were made in the Earle site because of extensive larval mortality by the nucleopolyhedrosis virus. In the Lakeview Drive site (total of 31,000 females released), 162 clusters were recovered from 1,576 larvae (10 percent parasitism). Ticehurst and Fusco (1976) released both *porthetriae* and *liparidis* in central and eastern Pennsylvania in 1975. Approximately 1,000 females of each species were released per 0.5 ha, although only five and one larvae were parasitized by *liparidis* and *porthetriae*, respectively. NJDA released approximately 5,000 female *A. liparidis* in 1977 in a 2 ha woodlot in Burlington County, N.J. The host population averaged 850 egg masses per hectare. Larvae were collected beneath burlap bands with peak percent parasitism by *A. liparidis* of 1.3 percent.

Brachymeria intermedia

This parasite was selected for inundative release because the species is easily reared in the laboratory and parasitizes the pupal stage of the host. NJDA released 45,350 adults (about 3,000 adults per week) in 1973 on one .04-ha site in Ocean County, N.J. The host population averaged 7,560 egg masses per hectare and 2,286 egg masses per hectare in the release and check plots, respectively. Pupae collected beneath

burlap bands showed 19.6 percent parasitism by *B. intermedia* on the release site as compared to 1.8 percent in the control site. There were significant differences between sites for pupal parasitism by *B. intermedia*, although R. Balaam of NJDA stated that greater defoliation (80–100 percent) was evident on the release than on the control site (maximum 60 percent)—indicating significant differences in host population densities.

Grimble (1975a) released *B. intermedia* adults (4,000 females per plot) in four release plots in 1974 in Delaware County, N.Y. These plots were monitored in 1973 by collecting 5,000 gypsy moth pupae; no *B. intermedia* were recovered (although recovered in low numbers in adjacent woodlot). The host population averaged 452 egg masses per hectare and 9,884 egg masses per hectare in 1973 and 1974, respectively. In the release plots, *B. intermedia* pupal parasitism averaged 32 percent within about 604 m of the nearest release point and less than 2 percent at 1,268 m or farther from release points. No check plots were evaluated for comparison. Defoliation of the forest by gypsy moth in 1974 stimulated the significant increase in *B. intermedia* parasitism; without defoliation this parasite was absent from 1975 pupal collections.

Barbosa et al. (1978) recommend that inundative release of *B. intermedia* be undertaken only on open-canopy sites or on sites in which dry, warm, bright conditions occur. *B. intermedia* may therefore be best adapted to use as a biological control in recreational areas, roadsides, suburban areas, etc.

Discussion

Parasite augmentation has not been shown to be an effective means of regulating populations of the gypsy moth in the Northeastern United States. Only three augmentation studies in the last decade reported significantly increased parasitism rates resulting from parasite releases in treatment areas as compared with control areas (Weseloh and Anderson 1975, Hoy 1975 b, Blumenthal et al. 1978). Of these, only the Eastford *A. melanoscelus* inundation study of Weseloh and Anderson (1975) and the *C. concinnata* inoculation study of Blumenthal et al. (1978) were conducted

within natural gypsy moth infestations. However, neither of these releases appeared to reduce significantly postrelease egg-mass counts.

Preliminary Attempts to Use Parasites in Combination With Pathogens in an Integrated Control Approach

Bernard J. Raimo and Richard C. Reardon

Introduction

Integrated control is one approach to pest population management that utilizes any combination of suitable techniques and methods of pest suppression in as compatible a manner as possible and maintains the pest population at levels below those causing economic damage (Smith and Reynolds 1966). Natural enemies are a logical component of such an approach, and their effectiveness can be enhanced in many ways: conservation practices for *established* natural enemies, use of selective chemicals, timing applications or reducing dosages of chemicals, etc. The integrated control approach has been used extensively in agricultural ecosystems (Allen et al. 1966, DeBach 1951, 1974, Falcon 1971, Gentry et al. 1969, Parker 1974, Stern 1969, van den Bosch et al. 1974) and less frequently in forest ecosystems (Coppel and Mertins 1977, Duylea et al. 1969, Maksimovic et al. 1970, Morris 1977, Morris and Armstrong 1975, Morris et al. 1974).

Several investigators have attempted to use combinations of biological controls to regulate gypsy moth populations (Wollam and Yendol 1976, Reardon et al. 1976, Raimo et al. 1977).

Aerial Application of Bacillus thuringiensis in Combination With the Release of Selected Species of Parasites

The bacterium *Bacillus thuringiensis* Berliner has been shown effective in causing mortality of gypsy moth larvae in the laboratory (Cantwell et al. 1961, Lewis and Connola 1966) and in the field (Dunbar and Kaya 1972, Dunbar et al. 1973, and Harper 1974).

In addition, *B. thuringiensis* is an effective foliage protectant (Lewis and Connola 1966, Yendol et al. 1973, Lewis et al. 1974).

By themselves, inundative releases of parasites against the gypsy moth have not resulted in immediate significant foliage protection or population reduction as determined by egg-mass density (see Parasite Augmentation). The objective of the combination was to produce not only foliage protection (afforded by *B. thuringiensis*) but also greater population reduction through the parasitization of the residual population.

Wollam and Yendol (1976) evaluated the microbial *B. thuringiensis*, the parasite *A. melanoscelus* (Ratzeburg), and the combination against the gypsy moth in Centre and Union Counties, Pa., in 1974. Twelve plots, each about 10 ha, were established and contained from 368 to 1,291 egg masses per hectare. The project comprised three treatments and a control, each replicated three times.

One treatment was a commercial formulation of *Bacillus thuringiensis*, Thuricide® 16B, aerially applied at the rate of 8×10^9 International Units (I.U.) per 0.4 ha. The first application was made when 50 percent of the gypsy moth larvae were in the second stage and when 50 percent leaf expansion had occurred on the white oaks. The second application was made 12 days later.

Another treatment was a release of the parasite *A. melanoscelus*. This species has been shown effective in low-density gypsy moth populations (Weseloh and Anderson 1975), and unaffected by *B. thuringiensis* sprays (Dunbar et al. 1973, Kaya et al. 1974). Approximately 1,000 mated females (from a laboratory colony from Yugoslavian stock) per treatment plot were released when most of the gypsy moth larvae were in the first or second stages.

A third treatment was the combination of *B. thuringiensis* and *A. melanoscelus*. Two aerial applications of Thuricide® 16B were made at the same rate and times described; about 1,000 mated female parasites were released in each treatment plot.

Treatments were evaluated on the basis of egg-mass numbers before and after treatment, relative density of larvae under burlap bands, drop-cloth collections, defoliation estimates, and parasite recovery.

In general, foliage protection and population reduction were not significantly different between plots treated with the parasite *A. melanoscelus* and the controls. Release of the parasite *A. melanoscelus* in areas treated with *B. thuringiensis* produced lower larval and pupal populations, less defoliation, and some reductions in egg-mass numbers. Where only *B. thuringiensis* was applied, variable results occurred, although the treatment provided foliage protection and population reduction when compared with the control or where only parasites were released.

Reardon et al. (1976) evaluated three parasite species (*Apanteles liparidis* Bouché, *A. melanoscelus*, and *Brachymeria intermedia* (Nees)) individually and in combination with *B. thuringiensis* against the gypsy moth. The study was conducted in 1975 on 27 blocks (21 treatments, 6 controls), each about 14 ha, of upland oak in Centre and Union Counties, Pa.

The first treatment was two aerial applications of Thuricide® 16B. Each application was made at the rate of 8 × 10⁹ I.U. per 0.4. The initial aerial application was made when at least 75 percent of the larvae were in the second and third stages and white oak foliage was 50 percent to 70 percent expanded. The second application was made 6 days after the first.

The second treatment was a combination of Thuricide® 16B, applied at the described rate, and 2,500 female *A. melanoscelus;* the third treatment was Thuricide® 16B and 5,000 female *A. liparidis;* and the fourth treatment was Thuricide® 16B and 2,800 female *B. intermedia*.

Treatments were evaluated on the basis of egg-mass density, egg viability, defoliation estimates, relative density of larvae and pupae under burlap bands, and larval mortality from 10-minute larval counts.

There were no significant differences in the numbers of viable eggs per mass among treated and control blocks. Results of egg-mass density surveys indicated that a spontaneous reduction in population had occurred in the entire study area. The population reduction had apparently occurred during the pupal stage, since control blocks showed 90 percent defoliation and no decrease in numbers of larvae. There were no significant differences among larval mortality

induced by the combination of Thuricide® 16B and parasites and by Thuricide® 16B alone.

Thuricide® 16B and Thuricide® 16B combined with a parasite provided excellent foliage protection. The foliage protection afforded by treatment with a parasite alone was not significantly greater than that of the control.

Prior to treatment, neither viable *A. melanoscelus* nor *A. liparidis* cocoons were found in the study area, although *A. melanoscelus* is known to be established in this area in Pennsylvania. Following inundative releases of these two parasites, an average of three *A. melanoscelus* cocoons per burlap band and 10 clusters of *A. liparidis* cocoons were recovered in the immediate vicinity of the release plots. These cocoons were heavily hyperparasitized. The initial high-density host population may have contributed to the failure of *Apanteles* spp. to provide significant foliage protection or population reduction. Although a substantial population reduction (posttreatment egg-mass density) was realized, this could not be attributed directly to the parasites. The initial high-density host population may have inhibited host searching by the parasites or parasitized hosts may have died from other causes, such as infections or noninfectious diseases. It was suggested that future releases of *Apanteles* should be attempted only where host densities are less than those in this trial.

In the *B. intermedia* and control blocks, 35 percent of the total pupal mortality was attributed to parasites. The chalcidid *B. intermedia* accounted for 45 percent of this total pupal mortality due to parasitism in the control blocks. In those blocks subjected to an inundative release of *B. intermedia*, this species accounted for 64 percent of the total pupal mortality attributed to parasitism. Since 45 percent of the total pupal mortality due to parasitism in the control blocks and 64 percent in the *B. intermedia* release blocks were attributed to *B. intermedia*, the high density of host population in these study areas must be considered a "favorable" habitat for the chalcidid parasite. Apparently, the incidences of tachinid and ichneumonid parasitism in the *B. intermedia* release blocks decreased, leaving the overall pupal mortality due to parasitism (35 percent) unchanged, so that

inundative release of *B. intermedia* caused no detectable decrease in total host population.

Treatment with Thuricide® 16B, 8 × 10⁹ I.U. per 0.4 ha, provided significant foliage protection. Thuricide® 16B was clearly responsible for the effectiveness of treatment when this agent was used in combination with the inundative release of each of three parasite species, because treatment with Thuricide® 16B plus a parasite was not significantly different from that of Thuricide® 16B alone.

Transmission of Nucleopolyhedrosis Virus by Apanteles melanoscelus (*Ratzeburg*)

Parasites and predators have often been cited as important vectors of insect pathogens (Magnoler 1968, Metalnikov and Metalnikov 1935, Smirnoff 1959, 1961, Smith et al. 1956, Tanada 1964, Thompson and Steinhaus 1950). One method of transmitting the disease from one host to another is by the contaminated ovipositor of a parasite. Thompson and Steinhaus (1950) showed that the ovipositor of *Apanteles medicaginis* Muesebeck, after it was contaminated by stinging a virus-infected larva, could infect a series of two of three larvae of *Colias eurytheme* Boisduval.

Current methods of applying microbial insecticides for gypsy moth population reduction are similar to those used in chemical control programs: topical applications of sprays and dusts (Grigorova 1962, Rollinson et al. 1965, Magnoler 1968, 1974, Cardinal and Smirnoff 1973). These methods when used alone may not be feasible for disseminating microbials, particularly virus, over large areas because of the current cost of their production and application. Also, in using conventional methods of application, a large amount of virus may be deposited on foliage that subsequently will not be eaten by larvae. Therefore, augmenting these traditional methods of dissemination with an effective means of transmitting the microbial from a focal point is desirable.

Reardon and Podgwaite (1976) demonstrated a positive correlation between the incidence of the nucleopolyhedrosis virus (NPV) and adult *A. melan-*

oscelus and *Parasetigena silvestris* (Robineau-Desvoidy) in natural populations of the gypsy moth in the Northeastern United States. Also, since the incidence of NPV increased with the larval instars, an increase in incidence for instars one through three should result in increased disease for that and subsequent seasons. Thus, *A. melanoscelus*, which parasitizes small instars, might be used to transmit NPV through several pathways: Direct mechanical transmission by insertion of a contaminated ovipositor; oviposition of a contaminated egg within the host; and indirect mechanical transmission by host feeding on foliage contaminated by contact with a contaminated parasite structure (for example, tarsi and/or mouthparts).

A. melanoscelus possesses several biological characteristics suitable for microbial transmission. It is distributed over a broad geographical range, it completes several generations per year, it reproduces by arrhenotoky, it is easy to rear in the laboratory in large numbers, and wild stock is readily available for incorporation into laboratory colonies (Reardon et al. 1973).

Raimo et al. (1977) conducted laboratory studies at Hamden, Conn., to demonstrate that *A. melanoscelus* has the ability to transmit NPV and successfully infect gypsy moth larvae, and to develop methods of contaminating parasites that could augment efforts to iniate epizootics artificially in gypsy moth populations.

Three methods of contaminating female parasites with NPV were tested: Manually contaminating the ovipositor of the parasite with virus suspension containing 1 × 10⁹ polyhedral inclusion bodies (PIB's) per milliliter; applying virus suspension (1 × 10⁹ PIB per ml) to the total body surface of the parasite; and exposing the parasite to infected first- and second-stage larvae when it was assumed that virus rods and PIB's were in the infected larva's hemolymph. In addition, one test group of parasites was not contaminated. The control group consisted of larvae that were not exposed to parasites. Host larvae were exposed to parasites for both 2 and 24 hours. Each test was replicated five times and continued for 30 days following initial exposure of the hosts to the parasites.

The incidence of mortality caused by virus in larvae exposed to contaminated and uncontaminated para-

sites for 2 and 24 hours was significantly higher than that in larvae not exposed to parasites (control). A significantly greater number of larvae died of virus when exposed to contaminated parasites than when exposed to uncontaminated parasites for 2 hours; however, there was no significant difference at the 5 percent level for 24 hours. The incidence of virus in all larvae exposed to contaminated parasites (both 2 and 24 hours) was significantly higher than it was in all larvae exposed to uncontaminated parasites.

The mean percentages of larvae dying of virus for the 2-hour tests were: 29.0 percent of larvae exposed to parasites contaminated topically; 38.0 percent of those exposed to parasites with contaminated ovipositors; and 16.0 percent of those exposed to parasites previously exposed to infected larvae. The incidences of mortality caused by virus were higher (not significant at the 5-percent level) for each of the three groups for the 24-hour test. Natural mortality caused by virus in larvae not exposed to parasites was 3.8 percent. Of larvae exposed to uncontaminated parasites for 2 hours and 24 hours, 9.5 percent and 10.0 percent, respectively, died from virus infection.

This laboratory study demonstrated that *A. melanoscelus* is capable of transmitting lethal doses of NPV to gypsy moth larvae. Data are currently being analyzed from laboratory investigations conducted to determine the exact mode of infection. Three modes of infection were possible: Intrahemocoelic inoculation of NPV inclusion bodies by means of the contaminated ovipositor; intrahemocoelic inoculation of NPV rods by means of the contaminated ovipositor; and the external contamination of gypsy moth larvae and/or gypsy moth diet by contaminated parasites. In addition, NPV did not appear to affect adversely the *A. melanoscelus* parasites, although Smith and Simeone (1977), using electronmicrographs, observed particles in *A. melanoscelus* cells that appeared to be gypsy moth NPV.

During 1976, a series of large (2×2×2 m) cages were located in a wooded area in an attempt to provide an "intermediate study" between the studies conducted in the laboratory and actual field release. Series of two cages were interconnected by hollow tubing to allow *A. melanoscelus* females, but not host larvae, to move between cages. Two treatments were attempted: First, 100 gypsy moth larvae were infected with NPV and placed in one cage with 20 uncontaminated *A melanoscelus* females and in the other cage were placed 100 healthy host larvae. For the second treatment, 100 healthy larvae and 20 *A. melanoscelus* females contaminated with NPV were placed in one cage and 100 healthy host larvae were placed in another. Two days after the parasites had been released into each cage, the parasites were allowed to pass to the other cage in the series. Unfortunately, too many *A. melanoscelus* females escaped from the cages or would not disperse through the tubes between cages. It is hoped that these cage studies can be repeated using cages of tighter construction and shorter interconnecting tubes.

Preliminary field testing of this integrated approach was begun when Wollam and Yendol (1973) demonstrated an increase in virus incidence due to the release of NPV-contaminated *A. melanoscelus* in Pennsylvania. During 1976, additional field testing was initiated, based on laboratory results whereby a small field test was conducted in northwestern Connecticut. Three blocks, each 0.5 ha, were established, and within each block five 0.04-ha plots were located. The gypsy moth population averaged 200 egg masses per 0.4 ha, and host larvae were collected from beneath burlap bands in the plots. Two treatments were made: 2,800 *A. melanoscelus* females contaminated topically with NPV solution containing 1×10^7 PIB per milliliter were released into each of two blocks and control.

There were no differences among the blocks for percent parasitism by *A. melanoscelus* (averaged 34 percent), while the *A. melanoscelus* release blocks averaged 39 percent NPV incidence, as compared to 22 percent for the control block. This increase in virus incidence was apparently due to the release of NPV-contaminated *A. melanoscelus* females.

In summary, some of the investigations have demonstrated that greater population reduction and foliage protection are afforded when pathogens and parasites are used in combination than when they are used individually. Laboratory studies where parasites contaminated with virus were evaluated for their

ability to infect gypsy moth demonstrated successful transmission. The exact mode of transmission, however, has not been determined. Results of preliminary field investigations using parasites contaminated with virus have demonstrated that this ability to transmit virus is not merely a laboratory phenomenon. Although the economic feasibility of this approach has not been established, the results of these attempts at integrated control should encourage more investigations of the relationships among the population dynamics of pests, parasites, and pathogens.

Summary

Richard C. Reardon

Biological control is the regulation by natural enemies of an organism's population density, at a lower average than would otherwise occur. All natural enemies are either parasites, predators, or pathogens. Both the classical and augmentation approaches of using parasites have been applied in an attempt to suppress populations of the gypsy moth.

The foreign exploration and importation program of exotic species has been one of the most massive efforts in biological control history. Between 1905 and 1914, gypsy moth immatures were collected in Europe, Japan, and Russia and forwarded to the Massachusetts laboratory for recovery of parasites. Six of the parasite species imported and introduced became established: Two tachinids (*Compsilura concinnata* (Meigen) and *Blepharipa pratensis* (Meigen)), one braconid (*Apanteles melanoscelus* (Ratzeburg)), one ichneumonid (*Phobocampe disparis* (Viereck)), one eupelmid (*Anastatus disparis*), and one encyrtid (*Ooencyrtus kuvanae*).

Parasite importation was reinstated in 1922 with searching for gypsy moth infestations in France, Spain, Italy, Germany, and Japan. The effort in Japan was discontinued following the 1923 season; the search for parasites continued in Europe until 1933. These efforts led to the definite establishment of two tachinids (*Parasetigena silvestris* and *Exorista larvarum*) and the possible establishment of one chalcidid (*Brachymeria intermedia* (Nees)).

During both periods of foreign exploration, 1905-14 and 1922-33, host and parasite collections were generally made from high-density gypsy moth populations.

Limited foreign exploration was resumed in the 1960's with numerous USDA-sponsored Public Law 480 projects and overseas contracts. In 1972, ARS established a gypsy moth project at the European Parasite Laboratory in France to recommence exploration and collection work in Europe. In 1975, ARS initiated studies in Japan and Korea with the opening of the ARS Asian Parasite Laboratory at Sapporo, Japan. Other than some small-scale work in France in 1976 and in Hungary in 1977, the ARS European Laboratory also turned its attention to Asia after 1975.

During the past 15 years, over 211,000 individuals of ±78 species have been sent to the ARS quarantine station in the United States from collection areas ranging from Morocco and Western Europe to Iran, India, Korea, and Japan. Very few "new" natural enemies of the gypsy moth were found during these recent overseas studies. Most of these were from Indian and Japanese "gypsy moths"; few currently show any great potential for effectiveness against the gypsy moth in North America.

Thus the classical approach to biological control of the gypsy moth (that is, the introduction of natural enemies) was successful in earlier attempts (1905-14 and 1922-33), but recent attempts to establish additional natural enemies have apparently failed. Possibly the U.S.S.R. and the People's Republic of China are areas where exploration may be of benefit; unfortunately, travel in these places is currently difficult.

The manipulation of parasites has not been shown to be an effective means of regulating populations of the gypsy moth in the Northeastern United States. Only three augmentation studies in the last decade reported significantly increased parasitism rates resulting from parasite releases in treatment areas as compared with control areas (Weseloh and Anderson 1975, Hoy 1975, Blumenthal et al. 1978). Of these, only the Eastford *A. melanoscelus* (Ratzeburg)

inundation study of Weseloh and Anderson (1975) and the *C. concinnata* inoculation study of Blumenthal et al. (1978) were conducted within natural gypsy moth infestations. However, neither of these releases appeared to reduce postrelease egg-mass counts significantly.

The use of combinations of natural enemies to suppress gypsy moth populations has been attempted in recent years: The combinations of the bacterium *Bt* and one of three parasites (*A. melanoscelus, A. liparidis* (Bouché), and *B. intermedia*), and NPV transmission by *A. melanoscelus*.

In one of the studies, *Bt* in combination with *A. melanoscelus* resulted in greater population reduction and foliage protection than when each was used alone.

In laboratory studies where parasites contaminated with virus were evaluated for their ability to infect gypsy moth, successful transmission was demonstrated. Results of preliminary field investigations using parasites contaminated with virus have demonstrated that this ability to transmit virus is not merely a laboratory phenomenon.

There is an obvious need for long-term intensive laboratory and field evaluations of individual parasite species as well as their complexes before effective utilization can be achieved for both established and exotic species. Several studies of individual species are currently being conducted in an attempt to identify various biological and behavioral characteristics of established species (*A. melanoscelus, B. intermedia, and B. pratensis*) and an exotic species (*Anastatus ?kashmirensis*) considered for introduction. On the basis of one of these studies it was recommended that *A. ?kashmirensis* not be released because of its anticipated ineffective role as a primary parasite; its propensity as a facultative hyperparasite; its morphological similarity with *A. disparis;* and its availability to attack other gypsy moth parasites after emerging from gypsy moth eggs.

This type of information on hyperparasitism, along with data on dispersal, alternate host requirements, and host and niche preferences, should provide the foundation for effective manipulation of parasites by themselves or coupled with other biological or chemical controls to regulate gypsy moth populations.

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6.2 Pesticides

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Introduction

From the time of its first general outbreak in eastern Massachusetts in 1889, the gypsy moth has confronted pest managers with an array of problems. In reaction to these problems during 80 years, pest managers have looked to pesticides as the primary prescription for combating gypsy moths. It is therefore appropriate first to discuss the problems facing today's pest managers and then to present the rationale utilized in evaluating the materials and techniques that attempt to alleviate these problems.

Problems

Gypsy Moth Biology

The single most confounding aspect of gypsy moth biology that affects control is prolonged egg hatch—2 to 3 weeks in any given area—which necessitates precise timing of ground or aerial applications and, in addition, requires a pesticide with a residual activity of 10 to 15 days. A biological pesticide lacking residual activity (as does *Bacillus thuringiensis*) may need to be applied twice, and this requires more time and more money.

Two other gypsy moth behavioral traits can at times cloud the process for selecting treatment areas or influence treatment success. These are windblown dispersal of first-instar larvae and migration of later instar larvae. If gypsy moth larvae are introduced by either means into areas harboring low-level infestations, unexpected pockets of defoliation can occur.

Pesticide Application

In addition to contending with gypsy moth biological aspects, the pest manager must also deal with numerous application problems. Selecting the appropriate delivery system to cut down on material loss due to evaporation or drift and to ensure adequate penetration of the forest canopy must be considered. Quality of application is very important, because it will determine treatment success or failure.

For example, skips in aerial spraying can lead to strips of defoliated trees in the year of application and leave population reservoirs that may result in premature population resurgence. Environmental factors must also be considered; even after the spray material has been deposited, biodegradation and washoff from rain may affect pesticide activity and, consequently, final results.

Superimposed over all these problems is the unpredictable and uncontrollable force—weather. In the Northeast, weather conditions as they affect gypsy moth development can cause a difference of as much as 3 weeks in initiation of suppression activities from year to year and from area to area within the same year.

In addition, weather conditions can significantly affect the duration of larval stages. For example, during cold, rainy weather a larval instar may be prolonged over a week but during hot, dry weather may be completed in several days. This kind of sporadic development complicates control program timetables.

Population Prediction

The inadequacies of various population survey techniques complicate the selection of treatment areas, particularly if suppression resources are limited. Since the state of the art relative to predicting rapid population declines is not wholly reliable, pest managers frequently find themselves in a spray-versus-no-spray dilemma for areas of potential population collapse. Pest managers have traditionally treated such areas anyway; the consequences of being wrong about the fate of the area were just too great to leave to chance.

There is evidence, however, that an outbreak might be prolonged because of a pesticide treatment (Doane 1968). Factors contributing to such a population response could be the interruption of an epizootic or starvation-related collapse, so that its full potential is not exerted upon the gypsy moth population, or the treatment-related reduction of gypsy moth parasites and predators. In Romania it was observed that when DDT was used as a preventative control measure,

higher gypsy moth populations resulted the following year, because of the killing of beneficial insects (Mihalache 1973). Such responses could allow numerous larvae to survive, leaving a reservoir population that might lead to a resurgence in the treated area sooner than in areas collapsing by natural means.

Special-Interest Influence

Public controversy over to treat or not treat for gypsy moth has plagued pest managers since control was first undertaken. Antispray proponents were influenced considerably by Rachel Carson's book, *Silent Spring* (1962). Today's research priorities and operational programs are often shaped according to the opinions expressed and actions taken by today's more informed and environmentally concerned individuals and groups, as can be plainly seen by reading the subject areas in the gypsy moth 1973 Environmental Statement (U.S. Department of Agriculture et al. 1973). Some of the groups requesting to review the Environmental Statement included the National Audubon Society, Sierra Club, Environmental Defense Fund, and Izaak Walton League of America.

The question of aerial application of pesticides, currently the most effective and efficient means of gypsy moth control, is an example of environmental influence commonly expressed as differences in policy among States, and among government units within States. For example, as a direct outgrowth to public concern and opposition to aerial treatment of gypsy moth and elm spanworm, Connecticut established a State regulation in 1972 banning aerial application of pesticides for nonagricultural purposes—and made the pest manager's job that more difficult.

Pesticide Registration

The pest manager is presented with yet another problem: the gypsy moth is a forest pest and not an agricultural pest, and consequently the selection of pesticides is quite limited. A review of the registration process underscores the complexities of this limitation.

The development and registration of a pesticide in the United States are arduous and costly procedures-$10-$15 million. Historically, it has been the control of agriculture pests and not forest pests that has offered the chemical industry the profit incentives to develop and register new materials or new uses for existing materials. Many times the initiative to register a compound for control of a forest pest has been taken by Federal, State, or university personnel.

This time-consuming and costly labeling procedure can be minimized by utilizing existing agricultural use data of a particular material (toxicology, phytotoxicity, and certain areas of environmental chemistry), but additional data must then be collected on laboratory and field efficacy, environmental chemistry, and nontarget effects as they relate to application of the material over a particular forest type. Once this is completed, a petition can be made to the EPA to expand the labeled use of the material to a particular forest use.

Rationale

The first step in resolving some of the problems facing pest managers is to know the patterns of gypsy moth populations in this country. Infestations are classed into three distinct types: The generally infested area, the periphery of the generally infested area, and remote infestations. Each of these areas is unique and thus requires special operational considerations.

In the generally infested area, a majority of the organized suppression activities occurs as part of the Federal/State cooperative program. Through these programs, high-value areas and areas that threaten artificial spread (infested campgrounds) are treated (U.S. Department of Agriculture et al. 1978). Within this area there is also much gypsy moth control activity conducted by private citizens. Although the quantity of pesticides purchased and applied by individuals or private applicators is not known, it is certain that the amount of pesticides introduced into the environment by the private sector far exceeds what would be introduced through a well-coordinated

program—a result of larger doses per application through ground treatment, as opposed to aerial treatment.

Natural spread is the primary concern of pest managers in the peripheral areas. The selected tactics here can be quite different and often more intensive than in the generally infested area—for example, the edge of a previous year's infestation is intensively monitored for signs of population outbreaks and areas of high population that pose the threat of spread are treated. (See U.S. Department of Agriculture et al. 1978 for a discussion of a recent proposal to address the problem of natural spread.)

Remote infestations occur outside the leading edges of the generally infested areas and are created by artificial long-range spread of hitchhiking life stages. As in California, Wisconsin, Illinois, and Michigan, these areas gain public attention every so often. It is expected that such remote infestations will continue to occur and be detected. Eradication of remote spot infestations is essential if they are to be kept from increasing in size and coalescing with other areas, as was the case in Michigan.

Under the regulations of the Federal domestic gypsy moth quarantine, the Animal and Plant Health Inspection Service (APHIS) is charged with reducing the opportunity for the establishment of remote infestations. Through this program artificial spread is reduced by regulating the movement of high-risk commodities.

During the Expanded Gypsy Moth Program, an attempt was made to find new pesticides that were compatible with the control objectives in each of the three gypsy moth infestation areas. The major emphasis included laboratory, field, and pilot testing, environmental impact evaluation, and improvements in the formulation and methods of application and in evaluation.

The primary interest in expanding the list of available registered compounds was to offer pest management personnel and regulatory agencies a wider selection of unique compounds. This approach would allow a land manager to take into consideration the favorable and unfavorable attri-

butes of the various materials and select one or more appropriate to needs without compromising environmental concerns. The application and evaluation improvements will help pest managers increase the efficiency and sharpen the subsequent evaluation of gypsy moth suppression efforts.

History
Before DDT: 1890–1944

The first recorded use of a chemical to control the gypsy moth occurred in 1890 as part of a State of Massachusetts eradication effort. The material was an arsenical, Paris green (Forbush and Fernald 1896). The spraying of infested trees and other foliage with Paris green was also supplemented with other accepted control techniques of the era: Applying creosote to egg masses; burning infested trees and shrubbery and clusters of caterpillars; and banding trees with burlap and sticky materials to either trap the larvae or prevent their ascent of the trees.

By the end of the 1891 field season, however, it was evident that Paris green could not be used to eradicate the gypsy moth. Besides its poor efficacy, Paris green was often phytotoxic and was easily washed off foliage (Kirkland 1905). Another problem with the compound was the adverse public reaction that its use generated (as reported by Forbush and Fernald 1896):

Considerable opposition to the use of Paris green for spraying was manifested by many people living in the infested towns. A mass meeting of opponents of the spraying was held in Medford. One citizen, who attempted to cut the hose attached to one of the spraying tanks, and threatened with violence the employees of the Board who had entered upon his land, was arrested and fined. Others neutralized the effects of the spraying by turning the garden hose upon trees and shrubs that had been sprayed, and washing off the solution. The opposition to the spraying affected the results of the work unfavorably to a considerable extent.

Experimental work on new pesticides continued, and while an effective eradication material was not available in 1892, bromine and chlorine were found to be useful in destroying egg masses in hollow trees. The following year, a new compound, lead arsenate, proved efficacious in the field (Forbush and Fernald

1896). For over 50 years, this material was the standard pesticide in the Northeast for gypsy moth control.

In the early 1940's, gypsy moth eradication efforts were all but abandoned, primarily because of the inadequacies of lead arsenate and problems with spray application. Lead arseante had to be applied during the period from mid-May, when foliage was well developed, to mid-June, and late-stage larvae were not always susceptible to the pesticide. In addition to these limitations, lead arsenate was toxic to animals allowed to graze underneath recently sprayed trees. Moreover, the methods and existing equipment for applying pesticides at that time were inadequate for any large-scale control efforts. Gypsy moth control and eradication efforts were revitalized after the introduction of DDT and the advent of spray aircraft and mist blowers.

The DDT Era

In 1939, the insecticidal properties of a synthetic organic chemical, 2,2 bis (parachlorophenyl) 1,1,1-trichloroethane, were discovered. For convenience, this compound was called by an acronym, DDT, derived from its generic name of *d*ichloro-*d*iphenyl-*t*richloroethane.

In 1944, the War Department allotted approximately 45 kg of DDT for testing as a gypsy moth control agent. After World War II, extensive research was conducted to determine efficacy of DDT against the gypsy moth and to evaluate its impact on nontarget organisms. During the period 1945–58, DDT was viewed as a panacea to eradicate the gypsy moth, and aerial spraying efforts used this pesticide almost exclusively. In all, over 5 million ha of forest in nine Northeastern States and Michigan were eventually treated for gypsy moth (U.S. Environmental Protection Agency 1975). At its peak use, over 1 million ha of forest and forested communities were treated in the 1957 spraying programs of New York, New Jersey, Pennsylvania, Michigan, and the New England States.

DDT was available as a dust, oil solution, emulsion, or water-dispersable powder. However, most of the gypsy moth suppression work utilized the oil solution because of its long residual action and superior foliar coverage. Early formulations consisted of 0.45 kg DDT (technical grade), 0.47 l xylene as an auxiliary solvent, and enough kerosene or No. 2 fuel oil to make 3.79 l. This was later replaced by an oil or kerosene solution with 12 percent (by weight) DDT with an auxiliary solvent capable of preventing crystallization of the DDT down to a temperature of –6.7° C.

Several years of experience with DDT revealed that an application rate of 1.13 kg of the material per hectare gave both 100 percent gypsy moth control and minimum direct losses to other forms of wildlife. The results of spraying with less material per hectare did not give complete control in all instances (Nichols 1962).

The effect of DDT on gypsy moth larvae was rapid. It acted as both a contact and a stomach poison; larvae not killed by contact soon succumbed following ingestion of treated foliage.

The following statement by Nichols (1962) helps summarize the views of pest managers relative to the use of DDT.

The value of DDT in gypsy moth eradication is established by its use in Pennsylvania at intervals during the past 18 years. At the standard rate of application, 1 pound of DDT per acre, there has never been any larval survival; no area has ever had to be aerially sprayed more than once to eradicate the existing infestation.

During the late 1950's and early 1960's public concern grew over the use of DDT. The material was being described as a "dangerous substance which killed beneficial insects, upset the natural ecological balance, and collected in the food chain, thus posing a hazard to man, and other forms of advanced aquatic and avian life" (U.S. Environmental Protection Agency 1975).

In 1957, for example, after 1 million ha had been treated in New York State, DDT residues were found on forage crops and in the milk of cows that grazed on treated areas. When these DDT residues were found to be persisting for as long as a year, New York suspended gypsy moth eradication efforts. This suspension was in line with Federal and State laws. DDT tolerances for milk were set at zero by the then

Federal Food and Drug Administration and by health authorities in New York (Brown 1961).

Starting in 1958, DDT was phased out of cooperative control programs over a 6-year period. In December 1972, EPA cancelled most uses of DDT.

Current Control Practices

In 1958, a new material, carbaryl, was introduced. Under the trade name of Sevin ®, carbaryl was to replace DDT as the primary agent to control the gypsy moth. From 1962 to 1977, approximately 900,000 kg of this material were used by Federal and State agencies against the insect in the Northeastern United States (Cartier 1977).

During this period, attention shifted from the wettable-powder formulations of carbaryl for aerial application to the oil formulations. Sevin 4-Oil®, a dispersion of finely ground technical carbaryl in a nonaromatic, low-volatile oil, was introduced. Besides the easier handling, this formulation provided better foliar coverage and a longer residual life. For ground applications on shade and ornamental trees, Sevimol 4® was used. This material consisted of a micropulverized carbaryl pesticide in an aqueous molasses medium.

Today, Sevin 4-Oil® is generally used for aerial application at 0.45 kg active ingredient (AI) per 1.18 l per 0.4 ha. About .24 l of kerosene is used with each liter of Sevin 4-Oil®. Sevin 80S® and Sevimol 4® are most commonly applied by ground equipment. Dose (liters of material per hectare) and application rates (kilograms AI per hectare) vary depending on the type of treatment.

Carbaryl is highly toxic to honeybees, but domestic bee poisoning problems have been minimized through organized programs that inform beekeepers daily of spray operations, thus allowing hives to be removed from spray areas or covered temporarily. These precautions, however, obviously do not minimize the potential effects on wild bee populations.

In the late 1960's, a broad-spectrum organophosphate, trichlorfon (Dylox®), proved efficacious against the gypsy moth. By the early 1970's, two registered formulations of Dylox® had been used in operational gypsy moth programs. One formulation, Dylox ULV® (ultra low volume) was discontinued when it was discovered that it pitted acrylic automotive paint finishes. The other formulation, Dylox 1.5 Oil®, has been used quite extensively in State suppression programs since 1972 (U.S. Department of Agriculture et al. 1973, 1974, 1975, 1976, 1977).

The 1973 Final Environmental Statement (U.S. Department of Agriculture et al. 1973) summarizes the performance of trichlorfon in gypsy moth control programs.

Trichlorfon exhibits low toxicity for honeybees, ... and [predaceous ground beetles] *Calosoma* spp. It is toxic to sarcophagid and tachinid flies and certain hemipterous predators, but these insects return to pre-treatment levels within several weeks after application. Due to its low toxicity to bees, their colonies do not have to be removed from spray areas. Trichlorfon applied at rates used for control of the gypsy moth should not cause a significant effect on non-target insects.

During the early 1970's, experimental laboratory work was initiated with an organophosphate (acephate) and a growth regulator (diflubenzuron). Developed under the trade names Orthene® and Dimilin®, respectively, both materials were tested and registered during the Expanded Gypsy Moth Program. Each is discussed in detail in this section.

Since 1958 several other pesticides were developed and registered for use against the gypsy moth, but because the advantages of these do not differ appreciably from those just discussed, they are mentioned only briefly in other areas of this text. Two exceptions are biologicals, the gypsy moth nucleopolyhedrosis virus and *Bacillus thuringiensis*, which are discussed in chapter 6.3.

A discussion of pesticide history would not be complete without a mention of pyrethrins. These are a class of pesticides that occur naturally in certain flowers of the chrysanthemum genus. Today, pyrethrins are produced synthetically and have demonstrated excellent laboratory activity against the gypsy moth. In the field, however, these materials are extremely toxic to fish and degrade too rapidly in sunlight (in 1 to 2 hours) to be efficaceous in gypsy moth control programs. Research work will continue with some of the new and more stable synthetics, but

until the fish toxicity problem is solved, it is unlikely that any of these materials will be registered for gypsy moth control.

Pesticide Evaluation

Evaluating pesticides in a hardwood forest for gypsy moth control required the establishment of efficacy and nontarget assessment parameters to insure that adequate data were collected. The following are the documented methodologies for establishing these standards.

Efficacy

Prior to initiation of pesticide laboratory or field evaluations, gypsy moth program and Environmental Protection Agency personnel conducted an efficacy workshop in February 1975. The objectives of the workshop were to establish the minimum amount of efficacy data required to meet EPA standards for experimental use permits, temporary use permits and final registration, and to develop guidelines for procedures and methodologies to acquire those efficacy data.

Four factors established early in the workshop proved essential in guiding discussions and identifying workshop outputs:

• The burden of supplying the appropriate data to EPA for various types of permits or for final registration rests with those submitting the petition.

• The scientists working with a particular pest are best qualified to establish efficacy parameters for the pest.

• The need exists for standardizing efficacy data collection, so that results will be comparable geographically and from year to year.

• The extent of efficacy data required would vary, on the basis of the proposed use of the material.

Participants at the workshop established two distinct criteria to determine the effectiveness of treatment—foliage protection and population control. By definition, foliage protection is achieved when refoliation is prevented. Refoliation, specifically in oaks, occurs when a tree (or portions of it) is more than 50 percent defoliated. Population control is achieved when retreatment is not required the following year. It was agreed that there may be foliage protection without population control; however, for successful population control, foliage protection is assumed as a necessary component.

It was also established that only a minimal amount of efficacy data is needed to support a label statement of foliage protection, while considerably more data are needed to support a population control statement. For the latter, it would be necessary to collect data on the specific species or on the complex of species being controlled, on the level of control achieved, and on the effect of the pesticide on the second generation.

The development and recommendations for use of any pesticide follow established procedures. The materials are first tested in the laboratory under a variety of bioassay procedures. Materials found to be promising in the laboratory are then evaluated in small-scale field experiments that usually test several formulations, applications rates, and application methods under a variety of field conditions. Prior to operational use, a pilot project is conducted to evaluate a promising control material or strategy under operational conditions.

Laboratory

The screening of pesticides and their various formulations against the gypsy moth is the responsibility of the Animal and Plant Health Inspection Service (APHIS) Otis Methods Development Center, Otis Air Base, Mass. During the past 17 years, over 800 laboratory experiments have been conducted using several hundred candidate materials.

Topical, diet, and seedling tests are the three laboratory techniques that have been used to evaluate material effectiveness against the gypsy moth. These tests are indicators of the contact and stomach poison capabilities of candidate insecticides. In a contact test, material is deposited topically employing a micro-application device. This type of test can screen large numbers of compounds rapidly for contact effectiveness. Potential stomach poisons are evaluated by

introducing them into a synthetic diet. This diet and the insects are placed in containers; thus the insects have direct contact with the substance and also use it as a food source. The third and most frequently employed insecticide evaluation technique is the seedling test. In these tests, candidate compounds are sprayed directly onto tender oak foliage. Test insects are then introduced onto the treated foliage and allowed to feed under simulated field conditions.

The seedling test is the preferred technique because it best simulates field conditions and allows determination of whether the pesticide is a stomach poison, a contact poison, or both. Procedures for conducting and evaluating these tests were sufficiently standardized (McLane 1973a) and were not altered at the efficacy workshop.

For all three tests, each material is evaluated at various dosages to establish a dose-response curve. The material is then subjected to simulated rainfall and sunlight to establish weatherability. Materials selected for field evaluations demonstrate a high degree of activity against the gypsy moth, weather well, and present minimal safety and environmental concern.

Field

Efficacy evaluations in the field were based on a randomized design with individual treatments replicated not less than four times. Individual treatment blocks ranged from 14.2 to 60.7 ha. Factors influencing block size were objectives of the study, population quality and density, host type, physiographic features, accessibility, and the size of available contiguous infested forest land.

Efficacy data were collected on 0.04- or 0.01-ha plots within each block. These plots were randomly distributed, ranging in number from 10 to 20 per block, dependent upon block size. The following were specific variables monitored in the 0.04- or 0.01-ha plots.

Defoliation

The extent of defoliation before and after treatment was estimated visually (1 percent increments) for each

tree larger than 7.6 cm in diameter at breast height (d.b.h.). At the time of data analysis, broader defoliation categories could be established. The degree of foliar protection for individual treatment blocks was assessed by inspection of aerial photographs, taken in a fixed-wing aircraft equipped with small-format camera mount.

Egg-Mass Density

Initially, egg-mass density was determined before and after treatment by counting the number of egg masses visible at ground level (including litter, shrubs, live trees less than 7.6 cm d.b.h., and dead trees) and on live trees over 7.6 cm d.b.h. Starting in 1976, egg-mass density was determined using an improved sampling procedure employing fixed- and variable-radius plots (see chapter 3).

Egg-Mass Quality

To support population-density estimates derived from fall egg-mass counts, egg masses were collected the following spring for evaluation. At least six egg masses were collected from each plot, with equal numbers collected between ground level and 30 cm up, and from sites more than 30 cm above ground. Following a laboratory technique method similar to that used by Saufley (1972), eggs were examined to establish overwinter mortality from parasitism, virus incidence, and noninfectious disease.

Larval Density

Ten-minute larval counts made at 6-day intervals were employed to follow larval population trends throughout the feeding period. Although somewhat subjective, these counts were indicative of treatment effect on the larval population. Without these counts, all evaluation must rely on fall egg-mass counts. There is an inherent problem in this approach should a population collapse occur in the later larval stages or pupal stage after treatment and after the target insect's response to treatment. Fall egg-mass counts comparing treatment to check blocks may not reveal

treatment effect; yet a treatment response may have occurred prior to population collapse. Consequently, a potentially effective pesticide could be overlooked.

Spray Deposit Assessment

Monitoring spray deposition provides a measure of the quality of the pesticide application. Adequate assessment will indicate if the material is being applied evenly, if sensitive areas are being effectively avoided, and if the application equipment is properly calibrated. Spray-droplet size and coverage were determined from white Kromekote® cards placed along transect lines within each treatment block. Spray cards were analyzed according to the method described by Dumbauld and Rafferty (1977).

Nontarget Organisms

Field

Field evaluation of a promising pesticide includes both documentation of its effectiveness against the target pest and its impact upon nontarget organisms present during treatment. To document the impacts of pesticides on nontarget organisms, it is first necessary to establish the type of data required to support registration. In concert with the Environmental Protection Agency and the Thompson-Hayward Chemical Company, program personnel developed protocols for evaluating the impact of diflubenzuron on a forest environment. This task was accomplished by combining accepted evaluation techniques for the various nontarget organisms with an adequate sampling regime.

Guidelines were established for evaluating a materials effect on soil arthropods and microorganisms, aquatic macroinvertebrates, fish, birds, small mammals, and gypsy moth parasites and invertebrate predators. Also established was a residue sampling and analysis program to monitor dissipation, persistence, mobility, and bioaccumulation of a material in a forest ecosystem.

These guidelines were used to solicit proposals for studies that, when completed and published, provided the required environmental chemistry data needed for the rapid registration of Dimilin® against gypsy moth. In addition, these studies will serve as excellent references for similar endeavors in the future. Further information on the details of the guidelines, including collection, transportation, and storage of samples, can be obtained from *Evaluation of Dimilin Against the Gypsy Moth and Effects on Non-target Organisms, 1975* (U.S. Department of Agriculture, Forest Service 1975).

Laboratory

An environmental concern attached to the use of pesticides for the control of gypsy moth is the impact of the chemicals on parasites that at times aid in maintaining the pest population below the outbreak level.

Pesticides currently registered for use against gypsy moth are not known to present a serious hazard to parasite populations when applied in accordance with recommended procedures. Information for evaluation of the impact of a pesticide application on parasites is obtained under field conditions and is usually derived from comparisons of parasitism rates in the treated area with those of a similar nearby site. On occasion, parasites caged within the treatment area have yielded useful information, but the kinds of observations on parasite effects that can be made under field conditions are very limited.

The controlled conditions of the laboratory, on the other hand, permit testing for a variety of biological effects and make possible the determination of relative potential hazards of different types of pesticides to various parasitic species.

Studies by Respicio and Forgash (1977) have shown that the adult *Compsilura concinnata* (Meigen) is more tolerant than gypsy moth larvae to topical doses of acephate or carbaryl, whereas *Brachymeria intermedia* (Nees) is more susceptible than *C. concinnata* of the host larvae to these compounds. On the other hand, *B. intermedia* is more tolerant than the gypsy moth to trichlorfon, while *C. concinnata* is equal to the host in susceptibility. Although *B. intermedia* females are more tolerant

than males to carbaryl, acephate, or trichlorfon, there is no difference in toxicity for the *C. concinnata* sexes. *C. concinnata* is not affected when sprayed with acephate at simulated field application rates, but considerably lower dosages are highly toxic to both sexes of *B. intermedia*. Foliage deposits of acephate, on the other hand, probably do not constitute a hazard for either parasite, as both species tolerate 24-hour exposure to oak seedlings sprayed at recommended field rates.

Gypsy moth larvae parasitized by *Apanteles melanoscelus* (Ratzeburg) are considerably more susceptible than unparasitized hosts to carbaryl, indicating that the survivors of a carbaryl field application may be predominantly parasite-free larvae. This could result in the reduction of the numbers of second generation *A. melanoscelus*, which attack third- and fourth-instar larvae (Ahmad and Forgash 1976). *Bacillus thuringiensis*, on the other hand, is equally toxic to both parasitized and unparasitized larvae (Ahmad et al. 1978). Whether the carbaryl effect is typical of other chemical pesticides such as acephate and diflubenzuron is not known.

Sublethal (to the host) doses of carbaryl on or within gypsy moth larvae do not affect the development of *A. melanoscelus*, indicating that gypsy moths that survive field applications of carbaryl are suitable potential hosts for the parasite and probably for other species as well (Pollack and Forgash 1978). There is also some evidence that sublethal oral doses of trichlorfon in the host do not affect *A. melanoscelus* development (Bonavita and Forgash 1977). Unfortunately, moribund larvae and those killed by carbaryl are as attractive as healthy larvae to the parasite but are unable to sustain progeny to maturity; presumably this would occur with other pesticides as well. The impact of such unsuitable hosts on the total effectiveness of an *A. melanoscelus* population is not known.

Diflubenzuron, because of its unusual mechanism of action, is not toxic to adult insects but is known to interfere with egg hatch in some nonparasitic species (Moore and Taft 1975, Wright and Spates 1976). Progeny production in *A. melanoscelus*, however, is not affected by ingestion (0.013 PPM in honey) of

diflubenzuron (Granett and Weseloh 1975), and the pesticide does not interfere with the reproductive capacity of *B. intermedia* when the adults are sprayed at simulated field rates of application (Respicio and Forgash 1978). Higher doses, however, enable the development of multiple *B. intermedia* progeny from a gypsy moth pupa rather than the usual single parasite per host; the explanation of this effect is not known. The progeny from diflubenzuron-treated *B. intermedia* appear to parasitize in the usual manner, producing one offspring per host. Concentrations of diflubenzuron as low as 0.01 to 0.03 PPM in the diet of gypsy moths interfere with development of *Blepharipa pratensis* (Meigen), which is a parasite of later stage larvae. Since diflubenzuron has rather long persistence on foliage, residues of spray applications may be harmful to progeny of *B. pratensis* (Khoo and Forgash 1978).

Materials

Currently there are nine chemicals registered for use against they gypsy moth. These materials, representing four pesticide groups (carbamates, organophosphates, growth regulators and biologicals), come in a variety of formulations for application from the air, ground, or both (table 6.2-1). The background information for compounds like carbaryl and trichlorfon has been covered in detail in the "Final Environmental Statement on the Cooperative 1974 Gypsy Moth Suppression and Regulatory Program" (U.S. Department of Agriculture et al. 1974). The biological pesticides *Bacillus thuringiensis* and nucleopolyhedrosis virus are discussed in chapter 6.3.

The background information for acephate is currently documented (Lake Ontario Environmental Laboratory 1975, McLane 1973a, and Willcox and Coffey 1977a). In addition, a limited discussion is presented in this section. Diflubenzuron represents a new class of pesticidal compounds, the first registrational use of which was for the gypsy moth. Because it is a relatively new material, little technical information is readily available to the scientific community at this time; the following is a detailed discussion of background information.

Table 6.2-1.—*Pesticides currently registered for use against the gypsy moth*

			Method of application	
Common name	Trade name	Formulation	Aerial	Ground
Bacillus thuringiensis	Dipel®	W/P	x	x
		LC		
		SC		
Bacillus thuringiensis	Thuricide®	16B	x	x
		W/P		
Carbaryl	Sevin®	50W	x	x
		80S	x	x
		4 oil	x	x
		4 flowable	x	x
		5 aqueous	x	x
	Sevimol®	4	x	x
Phasmet	Imidan®	50W	x	x
Trichlorfon	Dylox®	1.5 oil	x	
		EC		x
Malathion	Malathion®	EC		x
Maralate	Methoxychlor®	EC 2		x
Diflubenzuron	Dimilin®	25 W/P	x	x
Acephate	Orthene®	75S	x	x
Nucleopolyhedrosis virus	Gypchek	S	x	

W/P = wettable powder
LC = liquid concentrate
SC = soluble concentrate
EC = emulsifiable concentrate

Diflubenzuron

Description

Dimilin® is the trade name of the insect growth regulator diflubenzuron, N-[[(4-chlorophenyl) amino] carbonyl] 2,6-diflurobenzamide. It is a nonvolatile, white crystaline solid with a melting point of 230° C and a molecular weight of 310.7. Even though relatively new to the chemical market, Dimilin® has been widely tested throughout the United States and Canada and in many foreign countries. Experimental code names, which were used in early tests, included TH-6040, Largon, PH 60-40, ENT 29054, and OMS 1804 (Thompson-Hayward Chemical Company 1974, 1976).

Mode of Action and Morphological Expression

Diflubenzuron is effective against the gypsy moth because of its inhibitory activity in chitin biosynthesis.

This material is not similar to juvenile hormone mimics, although both interfere with the growth process of insects. The current consensus is that diflubenzuron acts by inhibiting chitin synthetase, the final enzyme in the pathway by which chitin is synthesized from glucose. This hypothesis is supported when the action of diflubenzuron is compared to that of polyoxin-D, a known inhibitor of chitin synthetase. Both agents produce identical effects on insect cuticles (Verloop and Ferrell 1976).

The primary effect of diflubenzuron follows ingestion; for this reason the larval stages of target insects are the most susceptible. It is postulated, however, that other stages such as pupae and eggs might also be affected by carryover or prolonged contact with the pesticide (U.S. Department of Agriculture, Animal and Plant Health Inspection Service 1977). At the initiation of ecdysis, the cuticle of treated larvae is only partially formed and is improperly attached to the epidermis. The new cuticle cannot withstand the increased pressure during

ecdysis and/or cannot give sufficient support to the muscles involved in the process. This results in the inability of the larvae to cast their exuviae and gives rise to the characteristic physical appearance of diflubenzuron-killed larvae (fig. 6.2–1). These larvae subsequently die from a rupture of the new and delicate malformed cuticle or from starvation (Thompson-Hayward Chemical Company 1976). There is a delayed response to diflubenzuron by gypsy moth because of the material's unique mode of action. Affected larvae do not die until the first molt after treatment. This delayed response is evident when larval mortality-response curves for diflubenzuron,

acephate (a rapid-acting pesticide), and nontreated areas are graphically presented together (fig. 6.2–2).

Toxicology

The basic toxicity to both mammals and nonmammals and the metabolic fate of diflubenzuron have been extensively studied and subsequently reported. Gypsy moth program personnel assisted in the preparation of a document summarizing much of the pertinent information on toxicological effects and environmental impacts of diflubenzuron (Willcox and Coffey 1978). Studies of subchronic toxicity and biochemical aspects of diflubenzuron were also reported by Willcox and Coffey (1978).

Figure 6.2–1.—*Characteristic appearance of gypsy moth larva killed by diflubenzuron (Dimilin®).*
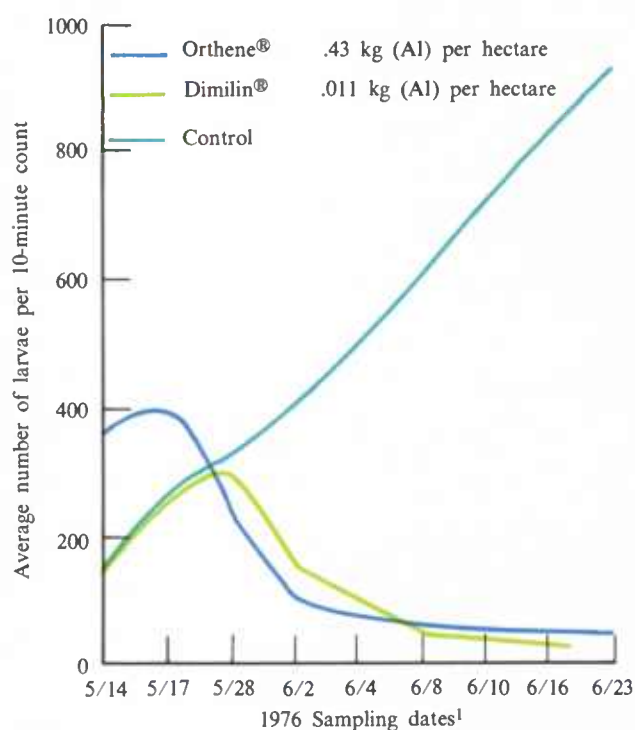
[1]Spray dates: May 16 for acephate; May 22 for diflubenzuron.

Source: Unpublished data on file with U.S. Department of Agriculture, Forest Service, Broomall, Pa.

Figure 6.2–2.— *Delayed mortality response of gypsy moth larvae to diflubenzuron (Dimilin®).*

From all of the studies conducted on diflubenzuron it is apparent that because of its unique mode of action the chemical is nontoxic to mammals, birds, and fish. The compound does, however, have an effect on some species of chitin-containing invertebrates. This response to the toxic substance varies a great deal among species and is related to the exposure level, the time of exposure, the ease with which the compound is absorbed by the organism, and its inherent ability to degrade the compound.

It is clear that diflubenzuron is rapidly degraded and that residue is eliminated from a number of domesticated animals and fish and does not accumulate within these species. The gypsy moth, however, does not possess the mechanism needed to detoxify diflubenzuron and, because of its sensitivity, is susceptible to very low levels once the material has entered its system (Willcox and Coffey 1978).

Fate in the Environment

Experiments in the laboratory indicate that diflubenzuron is neither persistent nor very mobile in soil. The short half-life of 0.5 to 1 week seems unrelated to soil type but dependent upon both the microbial activity of the soil and the particle size of the diflubenzuron. In the field, results indicate that after incorporation, at rates of 0.13 kg Al per hectare (0.3 PPM) into the top 7.6 cm of agricultural soil, the half-life of diflubenzuron was less than 48 hours. In addition, 4 weeks after completion, only 0.003 PPM leached into the 7.6- to 15.2-cm soil layer.

The half-life of diflubenzuron in water is only about 24 hours and is related to pH, temperature, and suspended organic matter in the water. In water-hydrosoil systems, the half-life is similar to that in soil, with the same qualifiers.

Studies introducing 1.0 PPM diflubenzuron into a closed aquatic system indicate that rainbow trout and bluegill sunfish accumulate levels 8 to 10 times higher than these in open water. However, 5 days after being placed in fresh water, the same fish had eliminated 90 to 98 percent of the accumulated diflubenzuron. Similarly, after higher initial accumulations, the level of diflubenzuron in aquatic plants decreased below that in the water after 2 to 4 days. The potential for bioaccumulation from an aquatic system exists because of the chemical structure of diflubenzuron. However, since diflubenzuron in actual field situations rapidly degrades and is subsequently absorbed by the soil and suspended organic material, the pesticide's bioavailability should actually decrease.

Finally, diflubenzuron is very stable following application on leaf surfaces. Research has shown that at 30 to 60 days after treatment, as much as 90 percent of the parent material could be detected. Detailed information on the persistence, mobility, and bioaccumulation of diflubenzuron in the environment, can be found in Willcox and Coffey (1978).

Efficacy Evaluations

Diflubenzuron efficacy evaluations were initiated in laboratory tests in 1973 and continued as various field evaluations for the duration of the Expanded Gypsy Moth Program. Work was also conducted on diflubenzuron outside of program funding, and findings from these studies are included in this discussion.

In initial laboratory efficacy tests, diflubenzuron did not show outstanding activity, mainly because tests were terminated before the material took effect. When tests were continued to allow the gypsy moth larvae to molt, the material looked excellent. In 1973 and early 1974, numerous dosages were tested ranging from 0.01 to 1.12 kg Al per hectare (McLane 1973b, 1974). The material was found to be efficacious at very low dosages, 0.01 to 0.07 kg Al per hectare. In the same laboratory tests, diflubenzuron also performed well when it was exposed to excessive amounts of rainfall and ultraviolet light. From these initial tests it was apparent that diflubenzuron should be taken to the field for further evaluation.

In 1974, the efficacy of aerially applied diflubenzuron was evaluated at rates of 0.13 kg and 0.27 kg (Herbaugh and McLane 1974) and at 0.45 kg (Sparnicht 1974). This same year, limited ground trials were conducted using a knapsack mist blower to apply dosages of 0.0018 to 0.057 kg Al per 37.9 l of water to individual apple trees (Granett and Dunbar 1975). Results were encouraging from these various tests.

Field evaluations of diflubenzuron intensified in 1975, with a variety of needed data being obtained. Aerial work was conducted at application rates of 0.067 kg AI down to 0.017 kg AI per hectare (Herbaugh and McLane 1975, Law 1975, Reardon et al. 1976); ground tests evaluated application rates of 0.028 kg AI down to 0.00028 kg AI per hectare (Granett and Weseloh 1975, Sparnicht 1975). Aerial treatments gave from 20-percent to 98-percent reduction of egg masses. The 0.067-kg treatment was made on 60.7-ha plots as part of a pilot study.

Laboratory tests continued in 1975 and on into 1976 to evaluate new formulations of diflubenzuron as well as to establish whether there was a need for a sticker (McLane and Finney 1976). It was concluded that a sticker is not needed when applying the wettable powder formulation (Dimilin W-25®) for gypsy moth control.

In May 1976, registration was granted for diflubenzuron applied by air and ground for control of the gypsy moth. Registration of the material and the problems related with its delayed action prompted a series of methods-development studies. These efforts were directed at further evaluation of treatment timing, application rates, and dosages (Herbaugh and McLane 1977a, Kegg 1977, U.S. Department of Agriculture, Animal and Plant Health Inspection Service 1977).

Two applications of diflubenzuron were made early in the 1976 field season using 0.067 kg AI per 9.35 l per hectare. One was applied to newly hatched larvae and the other 1 week later to first-instar larvae. Results were not good; heavy defoliation occurred in all plots. However, in most plots egg-mass reduction was substantial.

In addition, a new oil formulation of diflubenzuron (Dimilin 3.3 Flowable®) with various combinations of carriers and emulsifiers was field tested (Herbaugh and McLane 1977a; U.S. Department of Agriculture, Forest Service 1977). A 97-percent egg-mass reduction was achieved using a rate of 0.067 kg AI per 9.35 l per hectare.

Diflubenzuron wettable powder performed well in numerous field trials, as previously stated. By means of field days, presentations at meetings, and pub-

lications relating to diflubenzuron's effectiveness against the gypsy moth, the material was introduced to potential users. In 1977, diflubenzuron was used operationally on 1,733 ha in Pennsylvania and on 2.488 ha in New Jersey (Quimby 1977, Kegg 1977). When used in these operational programs, the material performed well during hot, dry weather, which induces rapid gypsy moth population development rates.

In 1977, the improved oil formulation, Dimilin OD-25®, was introduced. The new formulation provided excellent foliage protection and proved easy to handle (Munson and Reardon 1978).

Additional tests were initiated by scientists to establish an economical and satisfactory dosage for regulatory control personnel to use in treating remote infestations of gypsy moth (Herbaugh and McLane 1977b). Treatment effectiveness in these areas is considered successful only if gypsy moth populations are reduced below detectable levels. Final results revealed that a 100-percent reduction of egg masses could be achieved with two applications of diflubenzuron at 0.034 kg AI per 4.7 l per hectare. Even before the 1977 field season in the East was underway, diflubenzuron was utilized for treating an isolated infestation of gypsy moth in San Jose, Calif. (Romander 1977). The treatment appears to have been a success, since no male moths were trapped and no egg masses were discovered after intensive surveying.

In the same year, ground treatments using a mist blower were conducted to establish a standard rate of application for regulatory-related treatment in areas such as campsites and trailer parks (Herbaugh and McLane 1977b). Diflubenzuron was tested at application rates from 0.067 kg AI per 46.7 l per hectare to 0.00011 kg (AI) per 46.7 l per hectare. Egg-mass reduction was 100 percent with all treatments; however, defoliation averaged 80 percent in each 0.4-ha spray plot.

Although diflubenzuron is registered, more experimental work needs to be conducted with the pesticide. At present, most infestations are treated with two applications of 0.034 kg AI per 46.7 l per hectare. In 1978, tests will be conducted to evaulate

two treatments with lower application and dosage rates: 0.022 kg AI to 0.011 kg AI each in 4.7 l and 2.3 l per hectare. Single treatment tests will also be conducted with application dosage and rates as low as 0.011 kg AI per 1.17 l per hectare.

Acephate

Description

Orthene® is the trade name for acephate, an organophosophate pesticide containing O,S-dimethyl acetylphosphoramidothioate. It is a white crystalline solid with a melting point of 92° C to 93° C, a very low vapor pressure $(2 \times 10^{-6}$ mm Hg at 25 ° C, and a very high solubility in water (65 percent).

Mode of Action

Acephate, like all organophosphate pesticides, is a nerve poison—specifically, a cholinesterase inhibitor. Nerve impulse transmission is accomplished when a chemical mediator is released at one nerve ending to stimulate the next nerve. The mediator, acetylcholine, is then quickly destroyed by the cholinesterase enzyme, so that its "nerve firing" action does not persist. Acephate effectively blocks the cholinesterase enzyme, and as a result, nerve impulse transmission races out of control because of the buildup of acetyl choline at the ends of nerve fibers. The subsequent loss of motor coordination by the insect causes a reduction in feeding activity and a quick knockdown.

Toxicology

Like diflubenzuron, a great deal of time has been spent investigating mammalian and nonmammalian toxicity of acephate. Much of the pertinent information on the environmental effects of acephate has been published (Willcox and Coffey 1977a).

The metabolic fate of acephate in domestic animals has also received attention. Studies indicate that acephate does not concentrate in the flesh or tissues, including the fat of mammals, either during or after dosing. Animals eliminate acephate buildup in their bodies very rapidly. The main excretion route is via urine in mammals and feces in birds and most takes place within the first 12 hours, with a low level of elimination observed thereafter. Most of the remaining dose is found in the breath, with only traces found in the milk of cows and goats or in the eggs of quail and chickens.

Fate in the Environment

Prior to registration, studies were made concerning the persistence, mobility, and bioaccumulation of acephate in the environment. A brief summary of the existing data follows. Further information can be found in Willcox and Coffey (1977a).

Laboratory studies indicate that acephate is quickly degraded in soil. Half-lives ranged from 1.5 to 4.0 days in eight of the nine soils tested. Only in the very high organic-content muck did degradation exceed 1 week (13 days). Decomposition of acephate is primarily due to the action of microorganisms.

Although acephate has low soil persistence, it has relatively high soil mobility. The chemical is readily moved by soil water with little retention by the soil particles themselves. Residue percolation studies revealed that acephate ranging from 0.1 to 2.0 PPM could be found in ground water at the 0.3-m level after a 3.36 kg per hectare application. No residues were detected in any water or soil sample taken at 0.76 m or deeper.

Acephate degrades more rapidly in wet soil than in dry soil. For example, the half-life in sandy clay loam was determined to be 3 days if the soil moisture content is 6.5 percent and only 1 day if the solid moisture content is 20.3 percent.

In water alone, the half-life of acephate residues varies with water pH. At a constant 21° C, acephate half-lives were measured to be 55 days at pH 5, 46 days at a buffered pH 7, and 16 days at pH 9.

Acephate persists on leaf surfaces much longer than in water or soil. In studies using vegetable leaves, only an average of 5 percent of the applied pesticides could be washed off the leaves 14 days after treatment at 2.24 kg AI per hectare. There is only a slight systemic movement of the chemical from a treated leaf to other parts of the plant. However, acephate is readily picked

up by plants from treated soil and moved to the leaves during the transpiration process, where it accumulates.

Finally, as mentioned earlier, the studies on the fate of acephate residues in food-chain organisms and in model ecosystems indicate no bioaccumulation.

Efficacy Evaluations

Laboratory work was conducted with acephate in 1970 using a series of application rates from 0.13 kg AI per 9.35 l per hectare to 1.12 kg AI per 9.35 l per hectare (McLane 1973a). Results were so good that the material was subsequently field tested in 1971 and 1973 at application rates of 0.56 kg AI per 9.35 l per hectare and 0.14 kg AI per hectare (McLane et al. 1975, Secrest and McLane 1974).

In 1971 and 1973, a natural population collapse occurred making the interpretation of test results exceedingly difficult. However, results were promising enough to warrant additional field testing in 1974 and 1976 (Herbaugh and McLane 1974, Willcox and Coffey 1977b). In 1974 acephate was tested with propylene glycol as the carrier in one formulation and with water in another. Each formulation was tested at 0.56 kg AI per 4.7 l per hectare. Egg-mass reduction was 48 percent and 21 percent, respectively. When tested at 0.56 kg AI per 9.35 l per hectare in 1976 on second-instar gypsy moth larvae, acephate gave a 51-percent reduction in egg-mass deposition. Tests with propylene glycol revealed that the material offered little in the way of increased efficacy over acephate in water. In general, acephate gave good foliage protection in all tests conducted. Population reduction, based on egg-mass surveys, was not dramatic.

In May 1976, acephate was granted registration for aerial and ground application for control of the gypsy moth.

Selecting the Appropriate Pesticide

Problems that affect the quality of any control measure arise from areas such as biology of the insect, current spray-application technology, inade-

quacies in population prediction, changing tide of public opinion, and difficulty in registering new pesticides for forest use.

As mentioned earlier, one way to place these problems into perspective is to categorize gypsy moth infestations into three areas: The generally infested area, the periphery of the generally infested area, and remote infestations. It is apparent that each area has a different control objective and problems unique to the area. This situation creates special operational considerations and points out the need for individually considered control alternatives.

The general consensus among scientists planning the application phase of the program was that the needs of pest managers to overcome some of these problems could best be met in two ways. First, from previous experience with aerial spraying operations there was a recognized need for designing and testing technological improvements in both the support and evaluation phases of aerial spraying projects. The resulting improvements included a trailer-mounted pesticide mixing system, a radio communication network, and an aircraft mount that accommodates a small-format, inexpensive camera (White et al. 1978).

The second solution to these problems was to make available new pesticides that offered more qualitative advantages and fewer environmental impacts than the previously registered materials. Of the nine compounds currently registered, six are preferred for use in gypsy moth control programs. The current operational selection criteria for each of these six materials are presented in table 6.2-2. This table illustrates only information concerning the types of formulations available and whether or not a particular pesticide lends itself to a certain type of application, and not the advantages and disadvantages of each.

Acephate does not offer many more advantages than either carbaryl or trichlorfon. Diflubenzuron, however, is a unique compound that appears to possess greater qualitative effects than any of the other preferred materials (table 6.2-3). One possible advantage of diflubenzuron is that its registered application rate is about 33 times less than that of

Table 6.2–2.—*Operational selection criteria for preferred pesticides*[1]

Criterion	Diflubenzuron (Dimilin®)	Trichlorfon (Dylox®)	Carbaryl (Sevin®)	Acephate (Orthene®)	Bacillus thuringiensis (Dipel®)	Bacillus thuringiensis (Thuricide-16B®)	NPV (Gypchek®)
Registrant	Thompson-Hayward	Chemagro	Union Carbide	Chevron	Abbott	Sandoz	U.S.D.A.
Material class	Growth regulator	Organo phosphate	Carbamate	Organo phosphate	Bacterium	Bacterium	Virus
Active ingredient	Diflubenzuron	Trichlorfon	Carbaryl	Acephate	*Bacillus thuringiensis*	*Bacillus thuringiensis*	Nucleopolyhedrosis virus
Formulation							
Molasses dispersion			X		X	X	X
Oil dispersion		X	X				
Soluble powder			X	X			
Wettable powder	X		X		X	X	
Adjuvants							
Protectant							X
Spreader sticker	X		[2]X				X
Application							
Larval stage	1-3	1-3	1-3	1-3	[3]1-3	[3]1-3	[3]1-3
Number of applications	1	1	1	1	2	2	2
Aerial	X	X	X	X	X	X	X
Ground	X	X	X	X	X	X	X
Nozzles	Conventional (for example, flat fan) or power driven (for example, Beeco-mist®)						
Insecticidal activity							
Contact poison	X	X	X	X			
Stomach poison	X	X	X	X	X	X	X

[1]General use requirements for these pesticides can be obtained from their respective labels.
[2]For ground applications, stickers are normally used.
[3]Second application in 6 to 10 days, depending on larval development.

carbaryl or trichlorfon, and there are indications that even lower dosages may be effective. This certainly has positive implications for operational costs and the environmental burden. In addition to listing the positive environmental aspects of the six preferred compounds, table 6.2-3 highlights other possible attributes such as ovicidal activity and prebudbreak and population control.

One possible disadvantage of diflubenzuron is that it does not cause a rapid knockdown. Gypsy moth larvae, therefore, may continue to feed for as long as a week and could cause considerable defoliation before they succumbed. To be effective as a foliage protectant, care must be taken to apply diflubenzuron during early larval instars.

The known environmental impacts of these pecticides have been documented and should be considered in the selection process. If, for example, there are lakes, streams, and ponds located in a spray area, then a material known to have minimal effects on these can be selected.

Another criterion may be which of these pesticides best lends itself toward an efficient realization of a particular management objective for a spray area. In

Table 6.2-3.— *Comparative effects of diflubenzuron and alternative materials*

Characteristics	Diflubenzuron	Trichlorfon	Carbaryl	Acephate	*Bacillus thuringiensis*	NPV
				Treatment alternatives		
Operational performance						
RPAR candidate	X	X	X			
Tolerance established on agricultural crops		X	X	X		
Application rate (1 kg (AI) per hectare)	0.034–0.067	1.12	1.12	0.56	8 biu[1]	25 mpu[2]
No. of applications	1	1	1	1	2	2
Activity						
Contact poison	X	X	X	X		
Stomach poison	X	X	X	X	X	X
Rapid knockdown and mortality		X	X	X		
Foliage protectant[3]	X	X	X	X	X	X
Ovicidal activity	X					
Population control	X	X	X	X		
Pre bud-break control	X					
Fate in the environment						
Long persistence on foliage	X		X			
Short half-life						
Water	X	X	X		X	X
Soil	X	X		X	X	X
Environmental effects						
Adverse effects on nontarget insects						
Parasites and predators		X	X	X		
Pollinating insects			X	X		
Adverse effects on aquatic organisms						
Invertebrates	X		X	X		
Fish						
Temporary territory abandonment by birds		X	X	X		

[1] Bt is applied at 8 billion international units per acre per 0.4 ha per application.

[2] NPV is applied at 25 million potency units per 0.4 ha per application.

[3] Foliage protection would be achieved by definition when refoliation was prevented.

Source: Gypsy Moth Program Efficacy Workshop 1974.

residential areas, a compound that affords excellent foliage protection may be more desirable, regardless of how it affects subsequent gypsy moth populations. Conversely, in forest stands, a material that provides multiyear reduction in gypsy moth levels might be more desirable than a foliar protectant.

Since the discontinuance of DDT, carbaryl and trichlorfon have consistently attracted favor for use in gypsy moth suppression programs. Over this period, certain users have developed a preference for one chemical over the other in order to meet their objectives. For example, the State of Pennsylvania has traditionally used trichlorfon to treat large areas of infested woodlands; special interest groups, as well as the Pennsylvania Game and Fish Commissions, consider trichlorfon to be a safer material to apply in areas where streams, ponds, and lakes are present and have successfully effected the exclusion of carbaryl as a viable alternative compound. New Jersey, on the other hand, has developed a preference for carbaryl. This type of approach poses some serious limitations because the user may not achieve the desired management objective in the spray area; the use of one chemical to the complete exclusion of the other registered material may actually limit the overall effectiveness of the suppression effort.

Today, with an expanded array of compounds available, the challenge remains to convince users to abandon their preference for a single pesticide in favor of integrating these new compounds with respect to the impacts on the management objectives of those areas to be sprayed.

Of course, the actual selection of a particular pesticide often rests on the short-range cost of material and application. However, the availability of alternative compounds provides an opportunity to analyze the long-range benefits of using one pesticide over another, because each has distinct advantages. By recognizing the management objective of each proposed spray area the user can enlist the existing methodologies for assessing the socioeconomic impact of the gypsy moth. A subsequent benefit/cost analysis can be made that reflects the efficacy of each pesticide alternative, and a control option can be selected so as to maximize the long-term benefits.

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6.3 Microbials

Bacillus thuringiensis

Normand R. Dubois

Introduction

In the late 1950's and early 1960's, public pressure against the use of environmentally unacceptable chemical pesticides encouraged the investigation and development of biological agents, particularly entomopathogenic microorganisms, as alternatives for the control of the gypsy moth. One such microbial candidate was *Bacillus thuringiensis* (*Bt*), a spore-forming bacterium belonging to the genus *Bacillus* that during sporulation produces a toxic protein parasporal body called the delta-endotoxin (commonly referred to as the crystal) (figs. 6.3-1, 6.3.-2, and 6.3-3). Numerous species of Lepidoptera are susceptible to *Bt;* its pathogenicity against the gypsy moth was first reported in 1929 (Metalnikov and Chorine 1929). In 1960, Cantwell and his colleagues confirmed these earlier observations. They also observed that under similar field conditions, *Bt* compared favorably with chemical pesticide treatments for the suppression of gypsy moth populations and the prevention of excessive defoliation (Cantwell et al. 1961).

Early Research Activities: 1961–71

Field Application Studies

Studies on the aerial application of commercially prepared *Bt* formulations to control gypsy moth infested forest stands in the Northeastern United States were initiated in 1961. For the following 2 years, through 1963, successive improvements of the formulation and application techniques led Lewis and Connola (1966) to conclude that *Bt* could be used as a practical biological control agent against the gypsy moth. However, formulation composition and application schedules were important factors that affected the degree of control achieved. Specifically, the studies showed that the addition of a Baculovirus (gypsy moth nucleopolyhedrosis virus) to the *Bt* formulation did not enhance its effectiveness. Also, oils, commonly used as diluents for chemical pesticides, had an adverse effect on the insecticidal

activity of *Bt* when incorporated as the major carrier in the formulation. The use of oil with wettable powder formulations resulted in unanticipated physical problems of rapid settling in the mixing tanks and clogging of the spray nozzles during application.

With the development and use of a water-based liquid flowable formulation in 1963 (Thuricide 90 T® flowable), physical problems related to application were eliminated. However, the adjuvants used (stabilizers and emulsifiers) were feeding deterrents and also disrupted the physiological stability of the *Bt*, resulting in reduced pesticidal activity after storage for 20 to 30 days.

None of these three initial applications successfully reduced the larval population to the desired density of 50 egg masses per acre. Foliage protection, although temporary, was usually observed. More important, however, these initial studies provided impetus for the redirection of efforts to select more potent *Bt* strains and to add to spray formulations the necessary adjuvants that are chemically and biologically compatible with both *Bt* and the gypsy moth larvae. Others studying the feasability of using *Bt* to control this insect also met with variable success (Doane and Hitchcock 1964).

Continued effort to improve the effectiveness of aerially applied *Bt* through strain selection and formulation modification resulted in improved foliage protection and substantial though not acceptable (fewer than 50 egg masses per acre) population reduction. However, reliable recommendations aimed at further increasing its effectiveness could be made: Increase the spray volume (not necessarily the dose) to improve coverage particularly in thick canopied stands; use multiple applications at 7- to 10-day intervals to overcome the diversity of larval age, development rates, and susceptibility to *Bt;* and incorporate compatible adjuvants that will improved the sticking quality of the formulation on foliage, reduce evaporation during application, and extend pesticidal activity on foliage (Lewis and Connola 1965).

Implementation of these recommendations in a subsequent field test in 1966 showed that double application was more effective than a single

application of the same final dose per acre. Also, the addition of a sticker-antievaporant, Pinolene #1674 (Dubois 1965), to the formulation extended spore viability and pesticidal activity up to four times longer (4 weeks) than previously achieved (U.S. Department of Agriculture 1967).

These modest improvements in the use of *Bt* against the gypsy moth and other forest and agricultural insect pests stimulated its commercialization and expansion so much that the need for standardization of *Bt* products became acute. The development and acceptance of E-61 as the International Standard for *Bt* (Burgess 1967) somewhat alleviated this problem. E-61 is a stable formulation of *Bt* distribution by the Pasteur Institute (Paris, France) and defined to contain 1,000 International Units of Activity (IU) per milligram. By use of this universally available standard, comparison among *Bt* products became feasible regardless of strain or formulation differences. Soon all commercial formulations were labelled by IU as well as by the conventional viable spore counts (long recognized as an inadequate measure of pesticidal activity).

The isolation and commercial development of the HD-1 *Bt* strain (Dulmage 1970) increased by at least fifteenfold the pesticidal activity of *Bt* products over previously developed formulations. In May 1971, two commerical preparations of this strain (Thuricide HPC®, a liquid concentrate, and Dipel®, a wettable powder) were evaluated by mist blower ground applications in New Jersey and Massachusetts (Dubois et al. 1971). Three successive applications of

Figure 6.3-1.—*Vegetative cells of* Bacillus thuringiensis (*11 hours fermentation*).

8×10^9 IU per 0.4 ha each were made at weekly intervals beginning on second-instar larvae and when the foliage expansion on white oak was 50 percent. Both products effected excellent foliage protection (figs. 6.3-4 and 6.3-5). When the pest population was on the decline in Massachusetts, *Bt* increased the reduction, compared to the unsprayed areas; when the pest population was increasing in New Jersey, the rate of increase was greatly reduced. The estimated population reduction due to *Bt* was virtually the same, 85–86 percent regardless of which product was used.

Concurrently in 1971, ground application of *Bt* by mist blower was tested in Pennsylvania. A commercial formulation of *Bt*, Thuricide HPC®, was applied against gypsy moth larvae at two rates, 16×10^9 and 8×10^9 IU per 0.4 ha (Yendol et al. 1975).

Three applications of the lower dosage resulted in foliage protection and larval mortality. Defoliation approached 100 percent in the unsprayed areas and varied from 5 to 30 percent in the treated plots. The initial spray application when 50 percent leaf expansion had occurred may have been too late; better foliage protection might have been achieved if the material had been applied at 30–40 percent leaf expansion, with subsequent applications at 7- to 10-day intervals.

Laboratory Studies

The complexity of the mode of action of *Bt* against gypsy moth was first appreciated from bioassay studies on the spore-crystal-Beta-exotoxin complex

Figure 6.3-2.—*Sporulating cells of* B. thuringiensis (*24 hours fermentation*).

and on various strains of *Bt* (Lewis et al. 1964). Observations made in this study were that different strains of the same or different serotype groups (DeBarjac and Bonnefoi 1967, 1968) had significantly different potencies; two or more strains may express the same final mortality but differ in their rate of kill; spores contribute to the intoxication but are less effective than the crystal; and Beta-exotoxin blocks the molting process, and the stunted larvae eventually die.

With increasing interest in microbial control of pest insects, numerous strains and formulations of *Bt* became available for testing. Initially, 24 commercially prepared formulations were evaluated by bioassay for their pesticidal activity against the gypsy moth (U.S. Department of Agriculture 1964). Considerable variation in microbial potency was encountered, and several preparations were more potent than previously used field formulations. Bimodality in the mortality responses curve was consistently observed—a phenomenon that has never been satisfactorily explained. Also, storage temperatures adversely affected the stability of field formulations, but pesticidal activity was usually stable for 6 months if stored at 4°-6° C.

Variability was encountered not only with commercial formulations but also with newly isolated strains. Variations in laboratory assay procedures, growth fermentation conditions, and susceptibility within insect species of geographically different
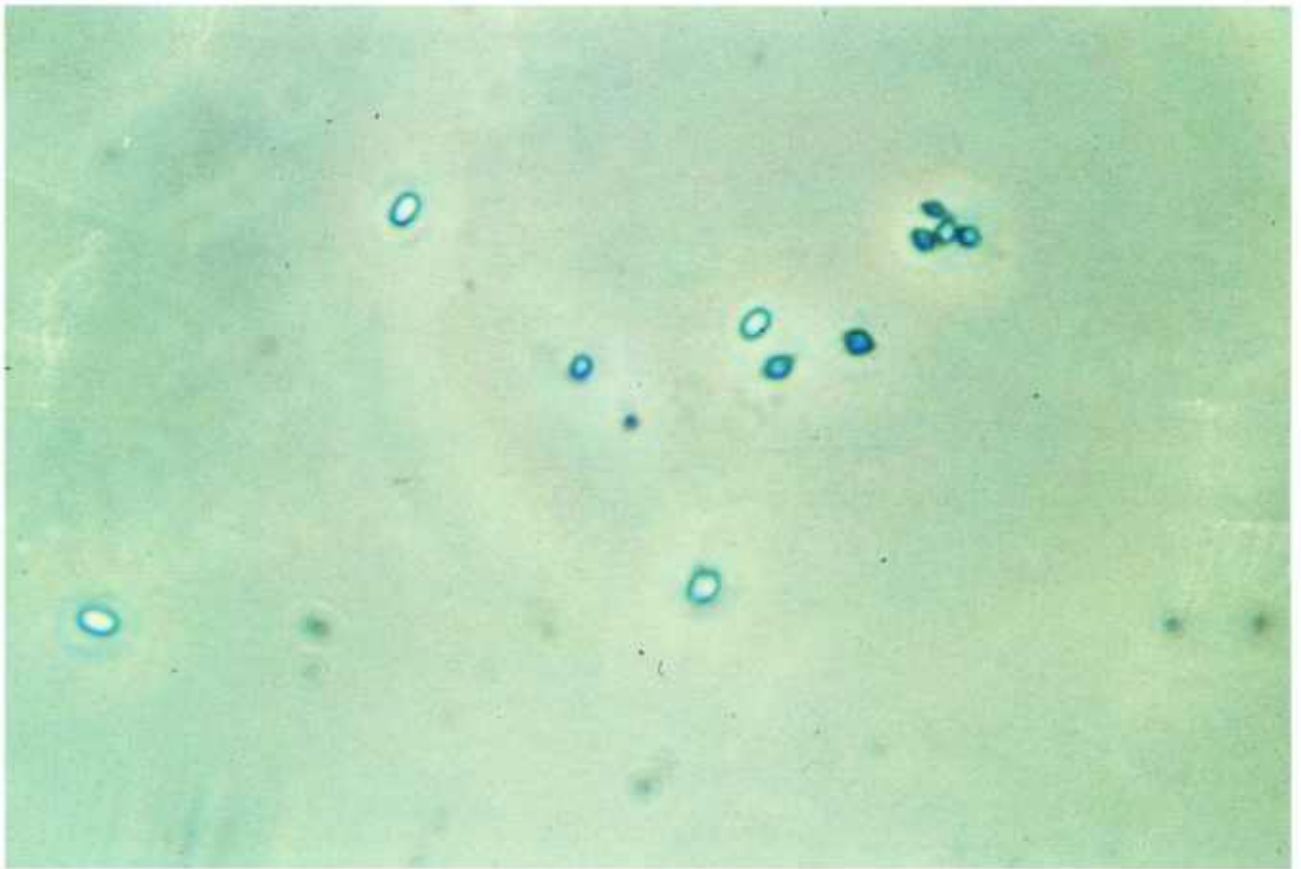
Figure 6.3–3.—*Spores and crystals of* B. thuringiensis (*40 hours fermentation*).

locales resulted in the proposal of several different *Bt* strains as the most effective against gypsy moth (Grigarova 1964, Herfs 1963, Ridet 1966, Ruperez 1967, Ruperez and Rossmore 1965, Vankova 1964). Even to date, laboratory examination of numerous strains indicates that there is no relationship between taxonomically related insect species and the effectiveness of specific *Bt* strains (Burgerjon and Biache 1967). Further, no relationship exists between the potency of taxonomically related *Bt* varieties and a specific source insect.

The development of E–61 reduced some of this confusion and facilitated the comparison of different strain against several insects. Using uniform growth conditions (Dubois 1968), Dubois and Squires (1971)

had found that taxonomically related *Bt* strains ranged over the entire spectrum of activity, and representative strains of several different serotypes were all equally potent. Furthermore, larvae of similar size and age in geographically distinct populations differed in their susceptibility to a given *Bt* preparation.

Accelerated Research Activities: 1971–78

Extensive fundamental studies necessary for the development of *Bt* as an alternative to chemical pesticides against the gypsy moth had been conducted prior to the 1972 gypsy moth program. However, application methods for practical and effective use of

Figure 6.3–4.—B. thuringiensis *treated block showing foliage protection against defoliation by gypsy moth* (*New Jersey, May 1971*).

Figure 6.3-5.—*Untreated area outside but adjacent to*
B. thuringiensis *treated plot in figure 6.3-4.*

Bt still needed to be refined and verified. Also, because bioassays remained the only reliable method of evaluating the pesticidal activity of *Bt* strains, an extensive screening program was maintained. Finally, the use of a specific enzyme to facilitate the invasion and infection of gypsy moth by *Bt* and other microorganisms was extensively investigated.

Laboratory, Field, and Cooperative Studies

Laboratory bioassays designed to determine the potency of *Bt* are usually conducted according to the procedures described by Dulmage et al. (1971). A bioassy procedure against gypsy moth larvae for preparations of *Bt* has been detailed by Yendol et al. (1973). In these bioassay tests, the commercial preparation of Thuricide HPC® resulted in an LD_{50} value of 96.2 1U per milliliter of diet when tested against gypsy moth larvae.

Feeding preferences have been conducted with gypsy moth in which larvae were allowed to select as a food source untreated or *Bt*-treated foliage (Yendol et al. 1975). Larvae were found to consume more of the untreated leaf disks or those sprayed with the lowest *Bt* concentrations. However, when molasses was added to the commercial preparation, consumption did not differ significantly between treated and untreated foliage. The incorporation of a feeding stimulant or masking substance obviously prevents

feeding descrimination, and the preferential selection of *Bt*-treated foliage could be important in field applications where spray coverage is not uniform.

In 1972 Lewis et al. (1974) evaluated five formulations of *Bt* against gypsy moth larvae in New York, New Jersey, and Pennsylvania. Two commercial preparations were evaluated, Dipel® and Thuricide HPC®, at 8 billion IU per 0.4 ha. Two gallons of finish spray per 0.4 ha were applied twice, about 10 days apart. Foliage protection was achieved in the New Jersey and Pennsylvania tests. The applications in New York showed no differences between treated and untreated areas, because of a population collapse due to a virus epizootic. In Pennsylvania, larval mortality exceeded 90 percent in all but one plot. Larval populations in the New Jersey plots resulted in 99 percent mortality because of a population collapse. In general, these applications did not result in adequate population reduction, and thus the residual egg-mass densities were sufficient to produce larval populations that would probably cause significant defoliation in 1973. In New Jersey, larval development was more advanced than desired and noticeable defoliation was observed before spraying was completed. Rain was extensive in the three States following applications; this may have also assisted in reducing the effectiveness of *Bt*.

Kaya et al. (1974) applied two formulations of *Bt*, Dipel® and Thuricide 16B®, and two synthetic pyrethroids by air in tests to control larvae of the gypsy moth. The Dipel® and Thuricide® preparations were applied at 7.26 and 8 billion IU per 0.4 ha, respectively. Foliage protection and the reduction of the number of egg masses were significantly greater in the treated plots than the control plots. However, the surviving population produced greater numbers of eggs per egg mass. Presumably the applied *Bt* reduced the larval population only just enough to avoid a potential stress situation that otherwise would have resulted in a high-density population producing smaller egg-masses. Consequently those surviving larvae were healthy and produced larger egg masses.

The synchronization of the application of *Bt* formulations with foliage expansion and the early larval stage is extremely important in maximizing the effectiveness of this microbial. In a review of the applications of *Bt* against gypsy moth larvae, Harper (1974) indicated that since 1959 some 14 different *Bt* formulations have been tried and they vary widely with respect to the additives. The variable results obtained in many of the investigations have probably been due more to the specific formulations used than the other factors such as timing or lack or potency.

There are a number of commercial formulations available, but extensive testing has not been done to evaluate their use in the control of the gypsy moth. Maksymiuk and Niesess (1975) investigated the role of various additives and the suitability of *Bt* formulations for aerial application against forest pests. These workers characterized and evaluated the physical properties of 12 water-based formulations prepared by two manufacturers. More of this type of testing is needed, in addition to the actual bioassays of foliage materials following aerial applications, to answer specific questions about formulations. Thorough coverage is extremely important because the material must be ingested to cause mortality. Feeding inhibition may also play an important factor in whether larvae consume a sufficient amount of the bacterium to cause mortality (Yendol et al. 1975).

Dunbar et al. (1973) had previously reported that aerially applied *Bt* had no apparent effect on three gypsy moth parasitoids, *Apanteles melanoscelus* (Ratzeburg), *Blepharipa pratensis* (Meigen), and *Parasetigena silvestris* (Robineau-Desvoidy). Later, Wollam and Yendol (1976) evaluated the individual and combined application of *Bt* and *A. melanoscelus* to reduce gypsy moth populations. Their results indicated that in those areas where the parasite was released, foliage protection and population reduction were not significantly different from the untreated areas. The aerial application of 8×10^9 IU per 0.4 ha of *Bt* provided foliage protection and population reduction. Some additional foliage protection was achieved where both biotic materials were used. This combination and the testing of other microbials and parasites need further evaluation under different gypsy moth population levels.

Continued Strain Selection and Evaluation

New strains of *Bt* are continually being isolated, identified, and made available for use; however, little guidance can be derived from the literature for selecting potentially effective strains against gypsy moth larvae. Therefore, bioassays still remain the most effective and reliable method for evaluating these untested strains and determining their pesticidal activity.

In an effort to determine the spectrum of activity of *Bt,* Dubois (1978) screened 350 isolates representing 14 serotypes against gypsy moth larvae. About 5 percent of the strains were considered very potent, 16 percent were grouped as moderately potent, 31 percent were grouped as weakly potent, and 47 percent were considered nonpotent. When individual isolates within the very potent and moderately potent groups were examined relative to the antigenic characteristics of their solubilized delta-endotoxins (that is, crystal), it was observed that some isolates produced serologically distinct proteins (toxins) common to four taxonomic varieties of *Bt* (that is, H varieties). Others produced toxins common to two H varieties, and still others produced delta-endotoxins specific to one H variety of *Bt*. If we assume that the antigenic properties of the delta-endotoxins are related to toxicity (Cooksey 1971, Rogoff and Yousten 1969), these observations may in part explain why several H varieties of *Bt* may have some very potent as well as nonpotent isolates. As a group, *Bt* variety H_{3ab} (Kurstaki) isolates having a crystal (delta-endotoxin) protein complex characteristic of the HD-1 type were the most potent. A proportionately high number of isolates of other H varieties having an HD-1 crystal type were also very potent.

Only a few representative isolates of any H variety, including H_{3ab}, that had a crystal type other than HD-1 were very potent against gypsy moth. It seems then that the protein complex of the crystal (or delta-endotoxin) may partially confer to an isolate the degree of potency it expresses. However, detailed studies on several H-variety isolates of different delta-endotoxins compositions at all levels of potency will have to be conducted before substance can be given to the above assumption.

Amplification of Insecticidal Activity of *Bt*

The addition of boric acid to *Bt* formulations substantially increased its insecticidal activity (Doane and Wallis 1964). Presumably the boric acid stressed the larvae so much that they succumbed more readily to the bacterial infection. Others (Morris 1977, Smirnoff 1973, Smirnoff and Valero 1972) have reported that chitinase combined with *Bt* enhanced its effectiveness against the spruce budworm, *Choristoneura fumiferana* Clem. In a preliminary study, Dubois and Gunner (1974) reported that bacteria isolated from healthy gypsy moth larvae became pathogenic to their host after growth in a chitinase inducing medium. Free N-acetylglucosamine was liberated from the infected larval tissue as a consequence of chitin depolymerization. They also found (Dubois and Gunner 1974) along with others (DeBarjac and Dumanoir Cosmao 1975) that numerous strains of *Bt* were inducible for chitinase.

In an extensive study on the pathogenicity of the microflora of healthy gypsy moth larvae after their induction for chitinase, Dubois (1977) found that numerous chitinolytic microorganisms could be isolated from healthy third-, fourth-, and fifth-instar larvae. The acquisition of the chitinolytic microflora appeared to be correlated with an increase of mobility by the maturing larvae. Selected isolates were inducible for chitinase not only by chitin but also by the insect-host chitinous tissue when used as the sole source of carbon in the fermentation medium. Stock chitinase (optimal pH at 5.2) produced by the isolates readily attacked the chitinous integuments of the larvae releasing *N*-acetylglucosamine. In vitro studies showed that the peritrophic membrane was very susceptible to the chitinase activity. In vivo studies demonstrated that two selected isolates were lethal to the larvae when fed as chitinase-induced whole cultures. The ingested chitinase caused localized dissolution and ulceration of the peritrophic membrane. However, the pH of the mesenteron appeared

to have a limiting effect on the in vivo chitinase activity on the peritrophic membrane. When chitinase was fed to larvae with sublethal doses of *Bt*, significant mortality was observed.

Summary

In presenting this account of the development and registration of *Bt* for use against gypsy moth larvae, little attention has been given to its advantages or limitations as an alternative to chemicals. With the increasing awareness of the impact of man's activities on the environment, stringent regulations are being placed on methods and agents used to protect forest stands from defoliating insects. In the light of these restrictions, *Bt* is a most attractive alternative to potentially nonusable and environmentally deleterious chemical pesticides. *Bt* is harmless to man, animal, and plant life, and it is selectively pathogenic against leaf-chewing insects such as the gypsy moth, but does not affect beneficial insects, including parasites and predators that may prey on the pest. Because of this selective pathogenicity, *Bt* is a unique insecticidal agent usable in an integrated pest management program where it can be combined with parasites, predators, pathogens, and other control techniques. As differentiated from chemical pesticides, development of immunity or acquired resistance to *Bt* has never been demonstrated in insects such as the gypsy moth. Once established in the host, it multiplies, resulting in lethal septicemia. Compared to other biological control agents, *Bt* is easily mass produced with currently available fermentation technology and equipment. The approximate purchase cost to the consumer, not including application costs, is around 50 cents per billion IU.

However, because *Bt* is a viable entity, its use can be limited. Its pesticidal potency as well as its viability can decrease rapidly in the environment after application; that is, climatic conditions will have a more pronounced effect on *Bt* than on a chemical. For this reason, use against insects with a staggered emergence and development such as the gypsy moth often requires multiple applications at about weekly intervals. Furthermore, its limited spectrum of activity may provide little foliage protection in a multipest infestation where some of the pest-insect species may not be susceptible to *Bt*. Finally, successful use of *Bt* requires more than just cursory knowledge of the insect. Recommended treatment time is usually related to some knowledge of the development, behavior, and or nutritional needs of the target insect.

Although *Bt* is registered against gypsy moth, research effort to reduce the variability of results obtained and increase its effectiveness should be continued. Screening studies indicate that some strains may well be more potent than the currently used HD-1 strain. Past changes of *Bt* field formulations were made to improve the spray delivery system and to extend pesticidal activity on the foliage. Now insect pathologists should also devote their research efforts to the optimization of in vivo solubilization and activation of the delta-endotoxin and to methods to facilitate the invasion of *Bt* into the host. Information derived from such studies could most certainly be used to increase the probability of infection when even small and variable amounts (sublethal) of *Bt* are ingested by individual larvae.

As more information about parasites, predators, and disease outbreaks is uncovered, their manipulation and use with *Bt* will undoubtedly be made in an integrated pest management program to suppress the gypsy moth. The successful development of such a program would have the advantage of not being restricted by environmental considerations such as pollution or elimination of necessary, beneficial insects.

It can be concluded that *Bt* is an effective alternative to chemical pesticides for suppressing gypsy moth populations and reducing defoliation damage. It can be used singly or combined in an integrated pest control program and is environmentally acceptable. However, for consistently effective results, formulation improvements should be made, and the user should be aware of its limitations and its vulnerability as a biological entity.

Gypsy Moth Nucleopolyhedrosis Virus

Introduction

Franklin B. Lewis

History

At the beginning of this century Reiff (1911) wrote, "I am quite convinced that we can apply the wilt in a systematic manner to the benefit of our forests, and that in so doing we shall come considerably nearer to a solution of the problem of destroying the gypsy moth". Here, in the last quarter of the 20th century, an attempt is still being made to cope with the gypsy moth problem and to make use of the wilt disease—nucleopolyhedrosis virus, or NPV.

NPV of the gypsy moth has been known by several names, usually derived from the appearance or behavior of the flaccid, sick larvae—*Flacherie, Flaccidenza,* or caterpillar cholera in Europe; wilt disease in the United States; and Wipfelkrankeit in Germany.

It is usually fourth-, fifth-, and sixth-instar larvae that are attacked by the disease, but diseased early-instar larvae, which are also susceptible, have been observed in the field. When epizootics of the disease occur in field populations, great numbers of cadavers can be observed.

The disease has characteristics that make its presence in gypsy moth populations readily apparent. Generally, virus-diseased caterpillars tend to climb upward on some object, die, and turn brownish black. Dead larvae hang limply in an inverted V position and have a shiny appearance (fig. 6.3–6). The cuticle is extremely fragile and will rupture at the slightest touch, emitting a brownish, foul-smelling liquid. Microscopic examination of this liquid reveals very large numbers of refractile polyhedral inclusion bodies (PIB's), about $1-10\,\mu$ in diameter, which contain the virus rods (fig. 6.3–16). The polyhedra produced in the nucleus of cells in the larvae give the modern name, nucleopolyhedrosis, to the disease.

This disease has been noted and studied as long as entomologists have been studying the gypsy moth. When the insect was accidently introduced into the United States and became a problem, the disease was observed and attempts were made to identify the causative agent and make use of the disease to control the insect (Glaser and Chapman 1913, Reiff 1911). However, it was not until Bergold's (1947) work that the true nature of NPV was known.

Largely because of the efforts of Steinhaus (1949, 1963), the entire field of insect pathology was established, and work in this area intensified. During this period, worldwide research on the virus disease of the gypsy moth similarly intensified (Doane 1970, Magnoler 1967, 1968a,b, Orlovskaja 1961, Rollinson et al. 1965, Wollam et al. 1978). NPV of the gypsy moth is receiving considerable attention in Europe, where several countries have joined forces to develop and produce the material as a control agent. An intensive effort is also ongoing in the U.S.S.R., where the gypsy moth NPV product is called Virion-ENSH.

Reason for Development

The gypsy moth is susceptible to a number of disease agents (Lewis and Etter 1978) occurring in nature. In addition, one of the important regulating mechanisms of the insect appears to be disease (Campbell 1963, Doane 1970, Podgwaite and Campbell 1971, 1972). Thus it was evident that disease agents, principally NPV, naturally affected the insect and, in many cases, appeared to cause a marked effect on the populations. NPV was therefore selected as one of two microbial agents (*Bacillus thuringiensis* was the other) for development within the gypsy moth program for the following reasons:

1. NPV is naturally occurring and has been implicated in causing natural collapse of the insect.

2. NPV is environmentally safe and, of the known natural pathogens of the insect, presented the least problems in registering a product based on the organism.

3. NPV is selective for Lymantriids only.

4. NPV gave evidence of carryover in natural populations, indicating long-term control potential.

5. Because NPV was a naturally occurring agent, it presented the possibility of enhancement or manipulation within the environment.

6. Laboratory evaluations and early field tests gave support to the use of the NPV as a direct control agent.

Figure 6.3-6.—*Gypsy moth larva killed by nucleo-polyhedrosis virus.*

The major thrust of the research and development activity with gypsy moth NPV has been to accumulate the necessary data to support Environmental Protection Agency registration of NPV for direct control use. NPV was also evaluated as a component of disease, as reported in chapter 4.

Laboratory Evaluations

Franklin B. Lewis, William D. Rollinson, and William G. Yendol

Introduction

Laboratory evaluations of the gypsy moth NPV were necessary to develop dose response data and to determine potency and the effects of storage on the virulence of NPV. These studies were necessary to calculate field-test dosages and to meet EPA registration requirements. Additional studies were conducted on the effects of selected pesticides on the activity of NPV and the effect of a mutagen on gypsy moth NPV.

There are a few terms used in this section that require definition: LC_{50}, LD_{50}, and LT_{50}. LC_{50} (lethal concentration) is the NPV concentration that, when fed to gypsy moth larvae, results in 50 percent dying of the virus. The measurement is the concentration of the NPV fed, with no measurement of how much is eaten. LD_{50} (lethal dose) is the actual amount of NPV consumed by the larvae that results in 50 percent dying. This measurement is the amount of NPV actually consumed. LT_{50} (time) is a time measurement based on either dose concentrations or actual dose

consumed and is the time (usually in days) required for 50 percent of the treated larvae to die. The 50 percent point is usually used because variation is the least at this point, whereas variation increases at the lower and upper (5 and 95 percent) points on the response curves.

NPV strain selection was quite difficult because several reports (Doane 1967, Magnoler 1968, Rollinson and Lewis 1973, Vasiljević and Injac 1973) indicated wide variation in the potency of NPV isolates tested against larvae of the same instar. A further complication was that different geographic populations of the insect responded quite differently to the same virus source. One to three log differences were reported in the LC_{50} of a given virus isolate tested against several gypsy moth sources. Similar variations were reported for several virus sources tested against a single gypsy moth population. These innate potency differences in the virus must be distinguished from the marked increased in the dose required to achieve an LD_{50} as the larval instar increases. Host susceptibility is confounded by food, population history, age, physiologic condition, and genetic constitution.

Table 6.3-1 illustrates the variation in activity reported in the literature. Table 6.3-2 illustrates the increased doses of the NPV needed to cause 50 percent mortality in second- through fifth-instar larvae.

Strain Selection

The first step in the development of gypsy moth NPV was the selection of the most virulent strain available. This required the evaluation of NPV material from around the world as well as isolates obtained from various sections of the United States. This selection was done to assure that the strain developed for registration was the most effective for insect populations in the Northeastern United States, and that the strain developed met identity criteria of EPA and was not a material of mixed heritage and potency.

The strain selected for development—the Hamden strain—was a compromise and generally exhibited stronger potency against U.S. populations. This strain

was isolated from a Connecticut population affected by a natural epizootic of NPV and has been used in all these field and laboratory studies. The primary gypsy moth NPV standard is the Hamden strain purified and concentrated by the procedures described by Breillat et al. (1972) and has been designated Hamden standard K-rotor. This standard preparation, thoroughly characterized, has been proposed as the International Standard. The preparation has been distributed to laboratories throughout the world and is available to laboratories desiring the preparation.

Bioassay Procedures

Numerous methods have been used to bioassay baculoviruses against Lepidoptera pests. Ignoffo (1964), attempting to establish the virulence of raw and purified suspensions of virus, used a method where the virus was incorporated into an artificial medium and then larvae were allowed to feed on the contaminated diet. Some investigators have used the surface-contaminated diet method (Burgerjon et al. 1975, Canerday and Arant 1968, Harper 1976, Vail et al. 1971) to study the infectivity of viruses. This was accomplished by inoculating the surface of the artificial diet with various virus concentrations and then allowing the larvae to feed freely on the diet. Concentrations were expressed as square millimeters of diet surface.

Another method involves contaminating the food source with a specific amount of the virus and then forcing larvae to consume the treated food and only selecting those larvae that have eaten all the treated food (Girardeau and Mitchell 1968). Baculovirus inocula have also been administered by the per os force-feeding method (Martignoni and Iwai 1977, Paschke et al. 1968). A specific amount of virus is fed to individual larvae with aid of a capillary tube and a microapplicator.

Two methods were developed for the gypsy moth NPV bioassay. The first, diet incorporation method, was devised to yield LC values and forms the basis for field formulations. The second, diet plug method, was devised to yield LD values.

Table 6.3-1.—*Variation in activity of gypsy moth NPV preparations reported by various authors*

Source of virus	Larval source	Instar (6 mg)	LC$_{50}$ PIB's per milliliter	Reference
Hamden K-rotor	Connecticut	II	5.8×10^5	Rollinson and Lewis (1973)
Hamden K-rotor	New Jersey	II	8.3×10^4	Rollinson and Lewis (1973)
Hamden K-rotor	Pennsylvania	II	2.4×10^5	Rollinson and Lewis (1973)
Hamden K-rotor	Michigan	II	6.5×10^3	Rollinson and Lewis (1973)
Hamden K-rotor	Massachusetts	II	4.5×10^3	Rollinson and Lewis (1973)
U.S.S.R.	New Jersey	II	2.0×10^5	Rollinson and Lewis (1973)
Japan	Michigan	II	4.3×10^6	Rollinson and Lewis (1973)
Yugoslavia	Michigan	II	3.5×10^7	Rollinson and Lewis (1973)
Connecticut	Connecticut	II	2.3 PIB's/ mm^2	Doane (1967)
Hamden K-rotor	New Jersey	II	473 PIB's/ insect	Rollinson and Lewis (1973)
Hamden K-rotor	Pennsylvania	II	220 PIB's/ insect	Hedlund (1974)
Yugoslavia	Serbia	II	3,200 PIB's/ insect	Vasiljević and Injac (1973)
Yugoslavia	Croatia	II	3,700 PIB's/ insect	Vasiljević and Injac (1973)
Yugoslavia	Slovenia	II	4,600 PIB's/ insect	Vasiljević and Injac (1973)
Hamden K-rotor	Yugoslavia	II	3,000 PIB's/ insect	Vasiljević and Injac (1973)
U.S.S.R.	Yugoslavia	II	5,100 PIB's/ insect	Vasiljević and Injac (1973)
Hamden K-rotor	Italy	III (23mg)	17,300 PIB's/ insect	Magnoler (1974)
Yugoslavia	Italy	III (23mg)	220,000 PIB's/ insect	Magnoler (1974)

Table 6.3-2.—*LC$_{50}$ values for Hamden Standard NPV against different instars of Pennsylvania strain larvae*

Instar	Average larval weight (mg)	LC$_{50}$ (PIB's milliliter of diet)
II	6.0	6.8×10^3
III	26.2	3.8×10^4
IV	101.0	8.6×10^4
IV	200.8	2.8×10^5

Virus Incorporated Diet Method

Four- to five-day-old gypsy moth larvae weighing 6 mg±2 are used in all bioassay tests (ODell and Rollinson 1966). A 10-mg sample of NPV powder is placed in a sterile tissue homogenizer with 10 ml sterile TRIS buffer (25 ml of 0.2 M 2-amino-2-hydroxy-methyl-1-3 propanediol mixed with 47.0 ml of 0.1 M HCl and diluted to 100 ml). The mixture is blended for 3 minutes; a 1:100 dilution is prepared from this stock suspension. Polyhedral counts of the diluted samples were made with an improved Levy-Neubauer hemocytometer. The charged chamber was allowed to stand for 10 minutes before the PIB's were counted at 440×. Twenty counts were averaged to estimate the number of PIB's per milliliter of the 10-mg sample. Five suspensions of decreasing concentrations (tenfold) were then prepared from the stock suspension. These calibrated suspensions were used to prepare the virus incorporated diet or the virus-contaminated diet plug for bioassay.

Table 6.3-3 lists the parameters used in the standard diet incorporated bioassay procedure.

Virus-Contaminated Diet Plug Method

Larvae of the same age and weight as used in the diet incorporation method are tested in the diet plug technique. Diet plugs are prepared by cutting into a 1-mm thick sheet of diet with a number 1 cork borer. These diet plugs are then placed in capped 28-g plastic creamers with a small wad of moistened cotton.

Various concentrations of the virus are prepared as described previously so that a single dose can be administered to the top of each diet plug. The virus dose is administered in 1-μl quantities to each plug using a microapplicator with magnetic stirring. The lethal dose determinations should be based upon 50 individuals for each concentration. Control groups are treated in a similar manner, except the virus is omitted and each plug is treated with 1 μl sterile water.

The bioassay insects are incubated at 24°–25° C under 16 hours of light.

As the diet plugs are consumed they are replaced with fresh, uncontaminated diet. Mortality is recorded for 14 days. Table 6.3-3 lists the essential parameters for this test procedure.

Concentrations of NPV assayed are expressed as polyhedra per milliliter of diet (diet incorporation method), nanograms NPV product per milliliter diet (diet incorporation method), and polyhedra per

Table 6.3-3.—*Test parameters of standard bioassay procedures for LC_{50} determinations and LD_{50} determinations*

Test insect	*Lymantria dispar*, New Jersey strain (F_{15}).
Larval weight and sex	Newly molted second-instar larvae (5–8 mg) both sexes.
Exposure to the virus:	
LC_{50} test	Virus incorporated into diet at 52° C.
LD_{50} test	Virus applied by microapplicator with a magnetic stirrer operating.
Inoculum volume:	
LC_{50} test	1 ml stock NPV to 99 ml diet. Two 1.25 cm³ diet cubes per sterile plastic petri dish (100×15 mm).
LD_{50} test	1 μl to a diet plug cut by a number 1 cork borer and trimmed to 4 mm in height.
Dose unit:	
LC_{50} test	Nanograms per milliliter of diet and polyhedra per milliliter of diet.
LD_{50} test	Polyhedra per diet plug.
Confinement:	
LC_{50} test	10 larvae per petri dish.
LD_{50} test	One larvae per 1 oz plastic creamer containing moistened cotton.
Group size	50 larvae per group.
Replication	One group per dose level, 5 doses.
Control	One untreated group.
Rearing temperature	24° C±2
Light regime	16-hour photoperiod followed by 8 hours of darkness daily.
Holding period	14 days (48 hours on virus diet).
Data evaluation	Berkson's logit chi square method (Paschke et al. 1968).

microliter (diet plug method). LC and LD_{50} are calculated by the Berkson logit technique (Paschke et al. 1968).

For additional consideration of virus bioassay concepts, international standards, and advantages and disadvantages of various techniques, consult the reviews of Dulmage (1973), Vail (1975), and Dulmage and Burgerjon (1977).

Establishment of Potency

The potency or activity of an NPV has classically been expressed as the concentration of PIB's per surface or volume. These figures have been used to construct dose response curves and also have been used in comparative potency determinations. This technique has serious inherent errors because the number and viability of the infectious unit are unknown (the occuluded virions). Dulmage and Burgerjon (1977) have explored this area. The Environmental Protection Agency (EPA) also addressed this problem by requiring that the labels of registered NPV products show a potency as well as PIB concentrations. To address this problem and to conform with EPA requirements, a gypsy moth potency unit (GMPU) was established.

The unit is defined as the weight of the product in nanograms that produces an LC_{50} in second-instar larvae as tested by the diet incorporation method. This is designated 1 GMPU. The standard LC_{50} bioassay (reported previously) is used and New Jersey (Otis strain) or Pennsylvania strain (lab) is the test (source) insect. The potency per gram, kilogram, or ounce is evaluated from the nanogram LC_{50} data. The New Jersey strain has been established as the standard test strain and is more resistant than the previously used Pennsylvania strain; therefore a conversion factor—2.989—has been calculated to convert potency units derived from the two strains. Thus the potency unit determined on New Jersey larvae is

multiplied by this factor to arrive at the potency unit in Pennsylvania larval terms.

Synergism of NPV and Chemical Pesticides

The NPV's of Lepidoptera are not as virulent as the NPV's of Hymenoptera. However, there is considerable evidence that the combination of sublethal concentrations of certain pesticides and microbials, including NPV's, increases the mortality due to the microbial alone. Benz (1971) has summarized the area of synergism of microorganisms and chemical pesticides. Chlorinated pesticides seem to have the greatest ability to synergize virus. O'Brien (1967) reported that these pesticides stimulate the microsomal enzyme system of living cells. Very little research has been done on organophosphate and carbamate pesticides as synergists for microbials.

The effects of certain pesticides as synergists for the gypsy moth were studied during this program. The Hamden K cleaned virus (Breillatt et al. 1972) was used in combination with candidate pesticides. The pesticides were applied topically at the rate of 0.1 ml on 1.5 cm^3 diet cubes (ODell and Rollinson 1966) at the start of each test and were tested for 4 days. The virus was incorporated into the diet at the stated concentrations and tested for 4 days. Normal diet was provided as needed for the remaining 10-day test period. Pesticide concentrations were made from wettable powders on the day of each test. Fifty newly molted second-instar larvae were used for each dose tested. The average weight of the larvae used was 0.0603 g (± 0.82 mg). Rearing temperatures were 24° C ± 2, with 12 hours of light daily.

Aldrin applied at the rate of 50 PPM had a synergistic effect concerning mortality, but antagonized the incidence of acute virosis.

Ignoffo and Montoya (1966) found that carbaryl lightly synergized the polyhedrosis of *H. zea* ($M_v = 61$ percent, $M_{v+i} = 79$ percent). *Lymantria dispar* larvae were fed sublethal doses (140, 80 PPM) of carbaryl

and NPV (3.7×10^4 PIB's per milliliter of diet), and a slight synergistic effect was found at certain combinations of pesticides and virus ($M_v = 44$ percent, $M_{v+i} = 58$ percent).

DDT at 10 PPM appeared to activate the incidence of polyhedrosis of *L. dispar*. An LC_{75} of 1.37×10^5 PIB's per milliliter of diet caused 78 percent virosis, and the same virus dosage with 10 PPM DDT applied topically caused 96 percent mortality of which 92 percent was polyhedrosis.

Dimilin at 1 PPM caused 82 percent mortality; however, Dimilin at 1 PPM and 1.0×10^4 PIB's per milliliter of *L. dispar* NPV gave 96 percent mortality of which 72 percent was virosis and an increase of 46 percent over the 1.0×10^4 PIB's per milliliter tested alone.

Work reported by Reichelderfer (1974) indicated that chemical mutagens would enhance the virulence of certain NPV's. A preliminary evaluation of this procedure was made to determine its feasibility with gypsy moth NPV. The tests reported here did not indicate that the technique should be employed with gypsy moth NPV because the increased virulence was not stable, was not as great as with other NPV's and finally the procedure could present difficulties in registering the NPV product.

The mutagen BuDR was layered on the surface of diet at the rate 0.5 mg per ounce of diet after incorporation of 2,400 PIB's per milliliter of diet. Virus was collected from the dead larvae and cleaned. The effect of the treatment was determined by bioassay, in the absence of the compound, and LC's were compared against those of untreated virus. This sequence was repeated three times. Larvae were tested in groups of 20 per petri dish, five replications for each treatment. Controls consisted of five groups of untreated larvae.

The first treatment of BuDR produced an additive and significant increase in virulence. This activity was assessed by passage after treatment and in the absence of the compound. This initial increase in mortality of 24.5 percent was followed by two successive decreases in the assay of the virus after BuDR treatment, while there were two successive increases during virus replication with the compound. If the polygenic control of virulence exists as suggested by Reichelderfer (1974), perhaps some of the mutations produced are lethal if the compound binds to or replaces specific nucleotides of the viral DNA and interferes with replication. Therefore, an equilibrium may have been achieved, as the third passage demonstrated, because virus mortality replicated with the compound and virus mortality replicated in the absence of the compound were about the same.

Reichelderfer reported that treated *Spodoptera frugiperda* NPV retained activity after 10 months at 4° C. The increase in gypsy moth NPV activity after 5 months at 4° C was 4.5 percent, a 21.5-percent decrease from the original increase of 26 percent.

The LC_{50} of the treated NPV after the first run was 2.4×10^3, compared to 8.6×10^3 for an untreated NPV LC_{50}.

This test was repeated using an LD_{22} as the initial inoculum. The NPV replicated with the compound was passed serially for five passes and tested four times in the absence of the BuDR. The first two passages showed a similar marked increase in the virulence of the NPV. The virulence then showed a decline similar to that recorded in the first test (table 6.3-4.).

Effects of Storage on Potency of Gypsy Moth NPV Preparations

It is important to know the conditions under which gypsy moth NPV preparations can be processed and stored to maintain maximum activity. Data of this kind are necessary for registration of the NPV product and also are important for the user to determine if storage conditions could lead to a significant breakdown in the product scheduled for field application.

Three forms of the NPV product were evaluated. Water suspensions, air-dried NPV powders, and

Table 6.3-4.—*Comparative mortality of* Lymantria dispar *NPV following treatment with BuDR*

Treatment	Percent mortality by passage number			
	0	1	2	3
Untreated[1]	30	26.5	24	30
During treatment with				
BuDR[2]	20	21.5	38.5	
After treatment[3]	—	51	48.5	36
BuDR alone	5	8	8	
Control	0	1	7	6

[1]Untreated virus passed serially at the LC_{30} dosage.
[2]LC_{30} dosage plus BuDR fed concurrently.
[3]Mortality obtained from PIB's recovered from treatment 2 used at LC_{30} dosage without concurrent BuDR.

lyophilized powders were stored frozen, at 4° C, at room temperature, and at 38° C.

Suspensions held at 4° C and room temperature showed no significant loss of activity for 2 and 5 years of storage, respectively. Suspensions held at elevated temperatures lost activity rapidly (within 3 months).

Air-dried NPV powder held frozen and at 4° C maintained activity for more than 1 year. Air-dried powder held at room and elevated temperatures lost activity rapidly (2 months and 2 weeks, respectively).

Lyophilized (freeze-dried) NPV material showed no loss of activity after 2 years of storage at 4° C.

In practical terms, storage of the gypsy moth NPV product would be best as a lyophilized powder stored at 4° C, an air-dried powder stored at –20° C, or a water suspension stored at 4° C.

NPV Production and Quality Control

Introduction
John D. Podgwaite

Production of gypsy moth NPV requires that the virus be propagated in either the living host or in an established cell line (in vitro). Although progress has been made in establishing a gypsy moth cell line for NPV production, at present the living insect is the only system available for economically producing the

amounts of NPV that are necessary to satisfy research and application needs. What follows is a history of gypsy moth NPV in vivo production techniques, from the very early use of naturally diseased, field-collected larvae, to the sophisticated state-of-the-art mass-production technology developed by the rearing team at Otis Air Force Base and discussed later in this chapter.
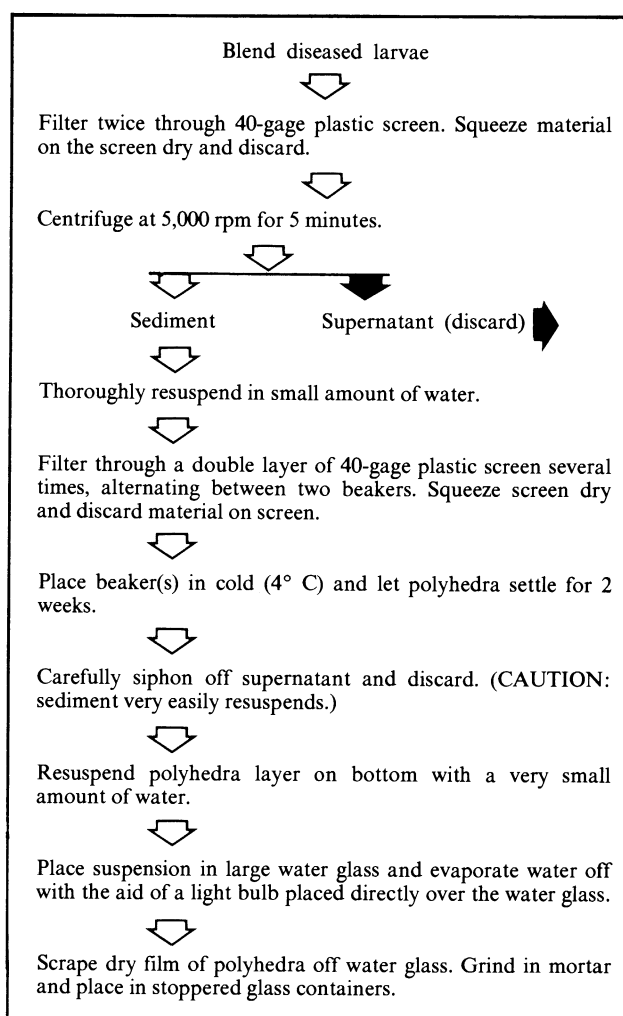
Figure 6.3-7.—*Scheme for the preparation of purified polyhedra powder* (Rollinson and Lewis 1962).

Early Production Techniques
John D. Podgwaite

When the potential of the gypsy moth NPV as a control agent was recognized in the early 1960's, it became clear that large amounts of the virus would be necessary for research and development programs. At that time registration requirements for microbials were not in place; in fact, it was not clear what safety and efficacy tests would be required by the responsible regulatory agency. Thus, it is not surprising that early NPV producers focused on maximizing the production of polyhedral inclusion bodies (PIB's) of the virus rather than producing a product that would meet rigid safety standards.

Pioneering production techniques (Rollinson and Lewis 1962) involved field collecting gypsy moth caterpillars from populations that were undergoing NPV epizootics. In heavily diseased areas, 1 pt of cadavers could be collected by one person per hour. This pint would then yield about 10 g of dried powder containing 6×10^{11} PIB's when processed according to the scheme outlined in figure 6.3-7.

Early production methods had several drawbacks. First, it was often difficult to locate NPV epizootics, and when they were found, they were not always convenient to the laboratory. Second, diseased insects were almost always dead for several days prior to collection and as a result were usually contaminated with opportunistic bacteria and fungi. Third, there was no practical way to insure that some of the insects collected were not killed by some pathogen other than NPV. Thus, early NPV production batches were often mixtures of many gypsy moth pathogens. Fourth, it was not possible to standardize either the virus (for identity and maximum virulence) or the insect (for optimal response to the virus). Finally, the methods were too time consuming, expensive, and crude for use in the production of large quantities of highly purified NPV.

To supplement NPV productions from field-collected insects, gypsy moth larvae were reared on NPV-contaminated foliage in the laboratory and processed as described. This was an improvement on the earlier method in that the insect could be standardized for response; however, the constant need for fresh foliage and the fact that the NPV dose ingested by each larva could not be controlled were serious constraints in maximizing PIB yield.

Microbiological quality control on early NPV productions was limited to standard plate counts. These were used to indicate the levels of bacterial contamination in the finished product. In the absence of guidelines, however, these counts were only useful in indicating the relative purity of the product.

Large-Scale Productions
John D. Podgwaite

Laboratory

The concurrent development and reporting of an artificial gypsy moth diet by ODell and Rollinson (1966) and Leonard and Doane (1966) had a major impact on NPV production methods. This allowed for the year-round rearing of the gypsy moth and the subsequent selection of strains suitable for maximizing NPV production. Further, it led to the development of the "diet incorporation" method of infecting larvae. This provided a technique for finding the minimum NPV dose that would yield the maximum number of PIB's from various larval stages.

Lewis (1971) reported on the mass propagation of gypsy moth NPV utilizing a laboratory-reared strain of gypsy moth and mass-rearing techniques developed by the Animal and Plant Health Inspection Service (APHIS) Otis Methods Development Center, Otis Air Base, Mass. Larvae hatched from surface-disinfected eggs were reared at 25° C in petri dishes and on artificial diet. Fourth-stage larvae were placed in pint containers and fed a diet containing 1×10^5 PIB's per milliliter. Virus-infected larvae were collected prior to death to yield 2×10^9 PIB's per larva. Using this technique, two to three people could infect and rear the larvae necessary to produce 10^{14} – 10^{15} PIB's per year. The cost for producing one larval equivalent (2×10^9 PIB's) of final product was between 2 and 3 cents. Since early experimental

NPV applications were made at the rate of 500 larval equivalents per acre, the cost of producing the NPV necessary for 1 ha (excluding formulation and application costs) was between $25 and $37.

Concurrent with the efforts being made to produce NPV more efficiently and economically came an increasing awareness of the potential hazards associated with the product—in particular its high bacterial load and the presence of allergens, primarily from gypsy moth setae. In the late 1960's, industrial groups were in the midst of developing *Heliothis* NPV for registration and were formulating, with the Environmental Protection Agency (EPA), safety standards for insect-virus preparations. In anticipation of stringent guidelines on product safety, the technique of isopycnic centrifugation in the zonal "K" centrifuge (Breillatt et al. 1972) was introduced for the purpose of removing allergenic particulates and unwanted biotypes from NPV preparations. This technique was quite effective in removing potentially dangerous particulates from the product, but its use increased the cost of producing one larval equivalent from 2 to 3 to 8 to 10 cents. This became an alarming $100-150 when translated to per hectare costs.

By 1972 Forest Service (FS) researchers had made substantive progress in developing NPV production technology. A Connecticut strain of the virus (Hamden standard) had been selected for use in future large-scale productions (Rollinson and Lewis 1973), and laboratory studies were continuing to find the best combination of NPV dose, larval stage, and method of infection that could be adapted to large-scale production methodology.

Microbiological quality control on early NPV productions was limited to standard plate counts. These were used to indicate the levels of bacterial contamination in the finished product. In the absence of guidelines, however, these counts were only useful in indicating the relative purity of the product.

One of the major objectives of the gypsy moth program was to support studies necessary for NPV registration. Thus, during the program several large-scale NPV productions were initiated to provide virus for efficacy and safety tests. A cooperative study with

W.G. Yendol, Pennsylvania State University, as well as contracts with R. Granados, Boyce Thompson Institute, and J. Simeone, State University of New York, were initiated and provided the NPV necessary for field and laboratory studies conducted by the FS from 1973 through 1975. All of these productions essentially involved scaling up, with appropriate modifications, the methods developed by FS researchers during the period 1962-72. At the time these contracts were initiated, difficulties were being encountered with rearing the laboratory strain of gypsy moth developed by APHIS. Therefore, larvae used for these large-scale productions were from eggs field collected from Pennsylvania.

Larvae were reared to the fourth stage on artificial diet and then fed a diet containing $0.3-1 \times 10^7$ PIB's per ml. Larvae were harvested when moribund, and PIB suspensions were prepared by a series of blendings, screenings, and differential centrifugations. These suspensions were further purified by zonal centrifugation in cooperation with J. P. Breillatt at the Atomic Energy Commission Laboratory, Oak Ridge, Tenn. Microbiological quality control and safety tests performed on the preparations obtained after zonal centrifugation indicated a product with a low bacterial count ($<10^7$ per g) that was free of allergens and mammalian pathogens.

Although the benefit/cost increase associated with large-scale rearings and processing was encouraging, the high, fixed cost of zonal centrifugation made the final product noncompetitive (on a per hectare cost basis) with conventional, chemical pesticides.

Commercial

In 1975 the FS contracted with Bio-Serv, Inc., Frenchtown, N.J., for the production of 5×10^{15} PIB's at a cost (exclusive of zonal centrifugation) of nearly 6 cents per larval equivalent. Technology used by Bio-Serv was essentially that of a commercial operation: Large-capacity rearing chambers, a refrigerated vacuum system for collecting cadavers, and large-capacity swinging-bucket centrifuges for concentrating PIB's. The final product was a PIB slurry ready for zonal centrifugation.

While Bio-Serv was proceeding with this contract, EPA released guidelines for safety testing and registering insect viruses (U.S. Environmental Protection Agency 1975). It became apparent after FS consultation with EPA that the zonal centrifugation step in processing would not be required for registration as long as the final NPV product met the prescribed safety requirements. At a Combined Forest Pest Program workshop in late 1975, a decision was made to eliminate the zonal centrifugation step in future NPV mass productions. This led to a series of modifications in the technology used by Bio-Serv so as to render a dry NPV powder with a bacterial load and other properties commensurate with EPA specifications.

The elimination of the zonal centrifugation step in NPV purification resulted in a more intense focus on the microbial quality of the less refined product. Microbiological quality-control tests were developed (Podgwaite and Bruen 1978) to monitor batch preparations of NPV for bacterial pathogens. These tests involved the examination of the NPV product not only for those microorganisms specified by EPA as not allowable but also for other potentially dangerous bacteria. They included an aerobic and anaerobic bacterial count per gram; a bacterial spore count per gram; a count of coliform bacteria per gram; fecal coliform detection; and the detection of the primary pathogenic bacteria *Salmonella, Shigella, Vibrio, Streptococcus, Staphylococcus* and *Clostridium.* Also, a mouse safety test, as required by EPA, was developed in order to determine the presence of toxic substances in the NPV product.

Early batch preparations of NPV produced by Bio-Serv contained no primary human pathogens; however, high levels of *Bacillus cereus* and its associated toxins were present. Because *B. cereus* and its toxins are pathogenic for mice, these early batches failed the mouse test at the required dose levels. The *B. cereus* contamination problem was traced to the NPV inoculum provided Bio-Serv by the FS as well as to contamination within the rearing facility at Bio-Serv. Intense efforts were then made to eliminate this microorganism from the NPV inoculum and to reduce its incidence to acceptable levels within the rearing facility. These efforts led to the lowering of *B.*

cereus levels to 10^5 per gram in a final dry powder that was free of human pathogens and elicited no mortality in mice.

The estimated cost of producing one larval equivalent of this product was about 2.5 cents. Based on this figure, the cost of producing enough virus to treat 1 ha at a dosage of 2×10^{11} was less than $12.50.

In late 1976, a joint FS–ARS–APHIS (Animal and Plant Health Inspection Service) research and development program on mass NPV propagation was initiated. The reasons for this were twofold. First, ARS–APHIS personnel were having success in mass rearing the colonized F_{13} New Jersey strain of gypsy moth; one of the major problems common to most previous mass NPV productions was the lack of a healthy, stable gypsy moth strain. Second, the elimination of zonal centrifugation from the NPV production scheme had left producers, such as Bio-Serv, unprepared to deal either with preventing contamination of the NPV product with unwanted biotypes, or the removal of these microorganisms from intermediate NPV products. Thus, the major objective of the FS–ARS–APHIS effort was to design and test a mass NPV production method (utilizing, where possible, clean-room technology) that would yield an NPV product meeting all the EPA requirements for microbial insecticides. This cooperative effort led to the development of the state-of-the-art gypsy moth NPV production methodology described in the following section.

In Vivo Production at Otis Air Base, Mass.
Martin Shapiro

In late 1975, research was initiated to optimize mass rearing of the gypsy moth. As a result of these developments, the impetus was provided for the initiation of a program to improve efficiency and develop technology for mass production of the gypsy moth NPV.

In early 1977, pilot plant production was initiated to produce virus from 500,000 insects. Based upon previous production (Smith et al. 1976), the total expected virus yield would approximate 5×10^{14} PIB's.

Materials and Methods

Initially, certain standards had to be established involving the test insect, the diet, and the virus inoculum. Thus, the colonized New Jersey F_{15} strain was chosen as the standard host insect. The modified hornworm diet (Otis production diet) was selected as the standard diet, and the Hamden isolate of *L. dispar* NPV was the virus standard.

The sequence of operations involved in NPV production is summarized in table 6.3–5. The scheme involved a single transfer of 14-day-old larvae (fourth instar) to a new diet. It would be desirable, for cost efficiency, if larvae could be maintained in the same containers throughout the production period. Preliminary data indicate the feasibility of this approach.

Much research was devoted to defining the parameters used for the production scheme, and the following is a summary of the elaboration of the scheme.

The Virus Inoculum

The virus inoculum is presented to the insect in association with food. Thus, the route of uptake of the virus is via virus-contaminated diet. The virus inoculum may either be incorporated into the diet during its preparation or added later to the surface of the prepared diet. From a practical viewpoint, much less inoculum is required to obtain a given level of virus yield when applied as a surface treatment.

In the production run, an inoculum dose of 5×10^6 PIB's per milliliter per cup was utilized. This dosage resulted in a maximal yield per larvae.

The Insect

To obtain a minimum yield of 1×10^9 PIB's per larva, it was necessary to inoculate larvae in the fourth or later instars. For virus production, a mixed population of male and female fourth-instar larvae was utilized.

For virus production, 10 fourth-instar larvae were maintained in a 180-ml cup. This number was chosen because growth and development were normal, and crowding was not considered excessive.

Environmental Conditions

For routine rearing, gypsy moth larvae are maintained at 25°–26° C at 50–55 percent RH. Although NPV yields and activity from larvae reared at 23°, 26° and 29° C were similar, the LT_{50}, or time required to kill 50 percent of the test larvae, decreased as the temperature increased to 29° C. At 32° C, however, both yields and activity decreased. Thus, 29° C was employed as the virus production temperature. The insects were held at a 16–8 light-dark cycle.

Harvest

In the standard NPV production schedule, larvae are harvested at day 10—that is, the time of about 30 percent mortality. Larvae were harvested at this time in order to minimize wilting of virus-killed larvae and loss of recoverable virus, and to minimize the bacterial load per larvae. The virus yield from day 10 harvested insects was comparable to that from larvae harvested on subsequent days (table 6.3–6). Both living-infected and virus-killed insects were frozen at day 10. Freezing prior to harvest was an advantageous and convenient method for inhibiting bacterial growth within insects, removing the larvae from the containers, and harvesting virus from wilted insects.

Table 6.3–5.—*Sequence of steps involved in production of gypsy moth NPV*

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1. Newly hatched larvae placed on diet (10 per 52-ml cup).
2. Larvae held from days 1–14 at 26° C.
3. At day 14 (14 days old, fourth instar), larvae transferred to 180-ml cup (10 larvae per cup) with 90-ml diet; surface of diet previously coated with 1 ml of aqueous suspension of virus inoculum (5×10^6 PIB's per ml).
4. Larvae on inoculated media held at 29° C for 10 days.
5. At day 24 (10 days post inoculation), containers with larvae place in freezer at –20° C; virus-induced mortality at 24 equals about 30 percent.
6. At day 25, frozen NPV-infected larvae harvested and returned to freezer until required for processing.

Table 6.3–6.—*Incidence of NPV-induced mortality and yield in relation to time of harvest for processing*

Time (in days) after inoculation	Percent mortality	PIB's per larva
6	0	1.30×10^8
8	1.4	1.05×10^9
10	31.7	1.75×10^9
12	81.9	2.07×10^9
14	97.8	1.53×10^9
16	100.0	2.07×10^9

Larvae and containers were placed in a large freezer ($-20°$ C) and left overnight. The following day, the frozen larvae (including those wilted) were easily removed. In 1 hour, 10,000 larve could be harvested by four persons. Following harvest, the larvae were placed in plastic bags, returned to the freezer, and held for subsequent processing.

Processing

For all processing (performed at Boyce Thompson Institute, Yonkers, N.Y., under the direction of Dr. H. Alan Wood), larvae were blended in distilled water in other to free the PIB's from host tissues. Following blending, the virus suspension was filtered through cheesecloth, and the filtrate was collected. Large tissue debris was retained by the cheesecloth. Since about 25 percent of the PIB's was also retained, the tissue "mat" was resuspended in distilled water, reblended, and refiltered.

In general, centrifugation has been used to concentrate the virus prior to air drying. Centrifugation, if it is to be economical, cannot be differential but must be carried out in a single run. The filtrate was centrifuged at 7,000 g per 25 minutes. Thereafter, the pellets were removed and air dried in a vertical laminar flow hood overnight under ambient conditions. The virus material was removed and was milled into a fine powder.

Results

Total production consisted of 502,500 larvae over a 47-day period. During 37 days of this period, samples of 30 larvae per day were taken and frozen. Weights of each group were taken and larvae were blended, and clarified, in order to determine total virus per group and virus field per larva.

The insects were inoculated with 5×10^6 PIB's per 10 larvae to give an expected LT_{30} 10 days later. The average mortality obtained for the run was 27 percent, well within expectations.

Data from the entire run are summarized in table 6.3–7. Two billion PIB's were obtained per insect, resulting in a total of 1×10^{15}. Smith et al. (1976) obtained about 9×10^8 PIB's per larva, utilizing late third-instar larvae. Program data indicated that larvae inoculated in the later instars produced more virus than those inoculated in the earlier instars (table 6.3–8). When fifth- or sixth-instar larvae were inoculated, it was possible to obtain greater NPV yields than with fourth-instar larvae.

To determine yields and potency of virus produced, 10 living-infected female larvae and 10 male larvae were collected each day during the production run. The greatest yields were obtained with females (tables 6.3–7 and 6.3–8), which undergo six larval instars, compared to only five instars for the male. A system wherein only females, or predominantly females, were reared and inoculated would allow a twofold to fourfold increase in NPV yield with little additional cost input. This prospect is being further investigated.

Although more research is needed, it was demonstrated that NPV can be produced for $1/1 \times 10^{11}$ and processed for $0.75/1 \times 10^{11}$, for a total of $1.75/1 \times 10^{11}$ PIB's (minimum dose per application).

Table 6.3–7.—*Yield of gypsy moth virus from pilot scale production run*

Total larvae inoculated	502,500
Total estimated PIB's recovered	1.07×10^{15}
Yield (PIB's per larva)	2.04×10^9
Range (PIB's per larva)	1.29–2.90×10^9
Yield per male larva	$\bar{x} = 1.57 \times 10^9$
Range	8.13×10^8–2.80×10^9
Yield per female larva	$\bar{x} = 3.99 \times 10^9$
Range	2.74×10^9–6.55×10^9

Table 6.3-8.—*NPV yield in relation to stage when larvae were inoculated*[1]

Instar	PIB's per larva
Second	3.96×10^8
Third	5.63×10^8
Fourth	1.40×10^9
Fifth	2.86×10^9
Sixth (females only)	4.9×10^9

[1]All larvae were virus killed.

Production in Cell Culture

James L. Vaughn, Ronald H. Goodwin, and Ellen Mika

Background

Viruses, unlike bacteria and fungi, are obligate parasites and can only replicate in the living cells of animals or in cultures of susceptible animal cells. The successful growth of animal cells in culture provided a major breakthrough in virology, and such cultures have been an important tool in virus research for years. In addition, cell cultures have had an important part in the commercial production of viral vaccines. The polio vaccine was the first to be produced in cell cultures, but methods are now available for producing vaccines against measles virus, adenoviruses, and foot-and-mouth virus in cell cultures. Thus, the instrumentation and methodology for the large-scale production of animal cells are being developed to meet the needs of medicine and public health. This technology can be divided into two types of culture: Those in which the cells grow attached to a surface of some type, and those in which the cells grow suspended in the medium. The cultures range from individual batch cultures to continuous cultures in which the virus and spent medium are removed and fresh medium and cells added automatically.

Among the advantages of cell cultures over animals are less handling, reduced contamination, greater purity of product, and better control of the environmental conditions during virus production.

Two of the several advantages of producing viruses in cell culture rather than in insects were especially applicable to the production of gypsy moth NPV: A cleaner product and stabilization of the virus.

Viral preparations produced from larvae contain large amounts of insect protein, cuticle, and hair. This material, highly allergenic to some humans, was removed from the virus by gradient centrifugation before the formulation of the final product. In contrast, virus produced in cell culture contains little insect material. Virus preparations from larvae also contain large numbers of bacteria and possibly other insect viruses in addition to NPV. Although cell cultures may also contain such contaminants, they can be maintained contaminant free much more easily and cheaply than living insects.

A second advantage of using cell cultures is the ability to stabilize the product. Available cell lines can be cloned and cell strains selected that produce a virus of high virulence and uniformity. Such cell strains can be stored frozen for long periods with little or no change. During production the conditions for cell growth and virus replication can be closely controlled to assure further a uniform, final product free of any microorganisms except the desired NPV.

Cell lines had been developed that could replicate NPV from several major crop pests (Hink 1972). Experience with production of NPV in these cell lines indicated that it might be feasible to produce the gypsy moth virus in cell culture if a suitable cell line could be found. In preliminary studies, cell lines available from *Trichoplusia ni, Spodoptera frugiperda,* and *Heliothis zea* were tested for their ability to replicate the gypsy moth virus. None of the available cell lines supported replication. This was as expected because of the restricted host specificity of NPV's.

Objectives

The project, as developed, contained the following areas of research: Establishment and characterization of cell lines from *Lymantria dispar* that would replicate NPV; formulation of a simple, inexpensive medium; development of volume culture methods;

and determination of procedures for obtaining the highest possible yields of polyhedra from cell culture.

Results and Discussion

Development of Cell Lines

The cell lines listed in the catalog prepared by Hink (1972) were derived from a variety of insect tissues such as embryos, hemocytes, and ovaries, but the most reliable source for cell lines from the Lepidoptera seemed to be the ovaries. Criteria for selecting ovaries for culture, based upon the degree of organ development, had been described earlier by Stanley and Vaughn (1968). The desired degree of development was one in which there had been extensive growth in organ size but little or no differentiation of ovariole muscle tissue and with few, if any, yolk-filled eggs. This development was reached in the early pupae of L. dispar; therefore, this stage was chosen as the source of tissue. Larvae were supplied when needed, either from the colonies at the Gypsy Moth Methods Development Laboratory at Otis Air Force Base or from Pennsylvania State University, and reared to pupation on an artificial diet.

For each primary culture, ovaries from 8 to 10 pupae were required. The pupae were surface sterilized by immersing them first in a 10 percent Haemo-sol® solution and then in hypochlorite solution. Dissected ovaries were treated with a 2.5 percent solution of collagenase followed by treatment with 0.1 percent hyaluronidase. This treatment loosened the cells of the component tissues but did not completely disaggregate them. The treated ovarioles were minced with sterile iris scissors. The tissue was pelleted by centrifugation, resuspended in growth medium, and transferred to culture flasks. Complete details of the primary culture methods are described by Goodwin and McCawley (1977).

After sufficient growth had occurred in the primary culture, the suspended cells and tissue fragments were transferred to a new flask along with half the culture medium. Fresh medium was added to the cells

remaining in the original culture flask. This procedure was repeated two or three times at weekly intervals and often produced sublines that differed in morphology and/or virus susceptibility (Goodwin et al. 1978). If the initial growth was rapid and quickly covered the original flask, the cells were removed by scraping with a rubber policeman, diluted with fresh medium, and transferred to new flasks.

The cell lines and sublines obtained from the gypsy moth are listed in table 6.3-9. All of the cell lines are identified in the standard convention: Letters identify the laboratory of origin, Insect Pathology Laboratory, Beltsville; letters identify species of origin, Lymantria dispar; and a series of numbers identifies each strain. These cell lines were serologically compared with each other and with the other lepidopteran cell lines in the laboratory as well as with pupal extracts from several insect species. Micro-

Table 6.3-9.—L. dispar *cell lines and their virus susceptibility*

Cell line and strains	Virus replication[1]	
	AC-NPV	LD-NPV
IPLB-LD-64:		
B	2	0
BA	2	0
BC	2	0
BD	2	0
IPLB-LD-65:		
CP	0	0
P	0	0
RQ	0	1
W	0	1
X	0	2
Y	0	2
Z	0	2
2	0	2
IPLB-LD-66:		
66	2	0
A	2	0
IPLB-LD-67:		
AB	2	0
AC	0	2

[1]AC-NPV = *Autographa californica* NPV; LD-NPV = *Lymantria dispar* NPV; 0 = resistant; 1 = some cells susceptible; 2 = all or most cells susceptible.

immunodiffusion tests showed that the new cell lines were not contaminated with cells from the other lepidopteran cell lines grown in the laboratory. It was further shown that all *L. dispar* cell lines had several major antigenic factors in common with the *L. dispar* pupae (Goodwin et al. 1978).

The susceptibility of the cell lines to NPV and the ability of the cell lines to replicate these viruses varied from fully susceptible (all or nearly all of the cells capable of replication) to partially susceptible (only a few cells in the line capable of replication) to resistant (table 6.3-9). Some strains of IPLB–LD–65 and IPLB–LD–67 contained cells that replicated the *L. dispar* NPV to the polyhedral stage. Cell lines IPLB–LD–64 and IPLB–LD–66 did not replicate the homologous virus. However, these cell lines would replicate the *Autographa californica* and the *T. ni.* NPV's. Two strains of IPLB–LD–65—65P and 65CP—would not replicate any of these viruses. Two other strains of IPLB–LD–65—652 and 65Z—were found to be composed of practically 100 percent gypsy moth NPV replicating cells. One of these, IPLB–LD–652, produced a large number of polyhedra per nucleus and was selected for further study in large volume cultures. All the cell lines that replicated the *L. dispar* NPV were also capable of replicating the *Orgyia pseudotsugata* NPV.

Media Improvement

The basal medium used in many of the studies on lepidopteran cell culture was that of Grace (1962), or some variation of it. This medium was compounded from chemically pure amino acids, organic acids, vitamins, and inorganic salts and was originally supplemented with insect hemolymph, which was eventually replaced with fetal bovine serum, bovine serum albumin, and whole-egg ultrafiltrate (Yunker et al. 1967). Although all these materials were available from commercial suppliers, the final medium was considered too costly for use in the large volumes required in the commercial production of gypsy moth virus. Therefore, efforts were made to develop a medium composed of less expensive materials. Early

studies had shown that satisfactory growth of primary cultures of lepidopteran cells could be obtained in a medium in which lactalbumin hydrolysate and TC yeastolate were used as replacements for the amino acids and vitamins (Jones and Cunningham 1961, Vago and Chastang 1958). Gardiner and Stockdale (1975) successfully adapted several continuous lines of lepidopteran cells to two media containing these hydrolysates and demonstrated that they could be used in large volume cultures for the production of NPV from the alfalfa looper, *Autographa californica*. The same hydrolysates were combined with Grace's medium to replace the bovine serum albumin and whole-egg ultrafiltrate used by Yunker, Vaughn, and Cory (Hink et al. 1974). These additives also permitted Hink and his co-workers to reduce the level of fetal bovine serum from 8 to 4 percent. Since the serum supplements amount to about 50 percent of the cost of the medium, this represented a major advance. Previous studies with vertebrate cells had shown that other hydrolysates or protein digests were useful in further eliminating the requirements for sera in cell culture media (Goodwin et al. 1978).

With this information in mind, several formulations were prepared and tested. Some were merely variations of the formulation used by Grace and were described as "amino acid based media." Others contained a balanced salt mixture similar to the Grace medium but contained various combinations of hydrolysates, peptones, and carbohydrates as the principal components; these are referred to as "peptone-based media." The most suitable formulations of each type are shown in table 6.3–10. These two media were the outcome of several modifications of a series of earlier media of each type. The earlier media have been described by Goodwin (1975) and Goodwin et al. (1978). The criteria used to evaluate each modification were maximum cell number, growth rate, survival times, and the effect on the production of gypsy moth NPV. Each medium was supplemented with 3 percent turkey serum, 3 percent chicken serum, and 3 percent fetal bovine serum. The turkey and chicken sera were toxic for these cells when used unheated, so all sera were heated for 60 minutes

at 60° C. These three sera in combination supported cell growth equal to that obtained in an earlier medium supplemented with insect hemolymph

Table 6.3–10.—*Media for the culture of* L. dispar *cell lines*

Component (mg per 1,000 ml)	Amino acid based (IPL–52) (mg)	Peptone based (IPL–73) (mg)
NaH$_2$PO$_4$·H$_2$O	1,160.0	2,000.0
NaHCO$_3$	350.0	.0
KCl	2,600.0	2,375.0
CaCl$_2$	500.0	200.0
MgSO$_4$·7H$_2$O	1,880.0	1,250.0
MgCl$_2$·6H$_2$O	.0	625.0
(NH$_4$) Mo$_7$O$_{24}$·4H$_2$O	.04	.030
CoCl$_2$·6H$_2$O	.05	.026
CuCl$_2$·2H$_2$O	.20	.032
MnCl$_2$·4H$_2$O	.20	.022
ZnCl$_2$	.04	.017
FeSO$_4$·7H$_2$O	.55	1.103
L-Arginine HCl	800.0	.0
L-Aspartic acid	1,000.0	.0
L-Asparagine	1,300.0	1,000.0
L-Cystine	100.0	.0
L-Glutamine	1,000.0	1,000.0
L-Glutamic acid	1,300.0	.0
L-Glycine	400.0	.0
L-Histidine	200.0	.0
L-Isoleucine	500.0	.0
L-Leucine	400.0	.0
L-Lysine HCl	700.0	.0
L-Methionine	1,000.0	.0
L-Proline	600.0	.0
L-Phenylalanine	1,000.0	.0
DL-Serine	600.0	.0
L-Tyrosine HCl	300.0	.0
L-Tryptophan	100.0	.0
L-Valine	500.0	.0
L-Threonine	200.0	.0
Hydroxy-L-proline	800.0	.0
Peptic peptone	.0	6,000.0
Lactalbumin hydrolysate	.0	4,000.0
Liver digest	.0	4,000.0
TC yeastolate	5,000.0	4,000.0
Glucose	5,000.0	7,000.0
Maltose	1,000.0	1,000.0
Sucrose	.0	2,500.0
Glycerol	2,700.0	2,700.0
Folic acid	1.2	1.2
Isoinositol	10.0	10.0
Cyanocobalamin	1.0	1.0
Acetyl-β-methyl-choline chloride	250.0	250.0
Polyvinylpyrrolidone	2,500.0	2,500.0

(Goodwin 1975). Typical growth curves for the IPLB–LD–652 line are shown in figure 6.3–8. The cells grew somewhat faster on the final amino acid based medium, IPL–52 (population doubling time, 75 hours) than on the peptone-based medium, IPL–73 (pdt, 107 hours); however, higher cell numbers were obtained with the IPL–73 medium. A combination of equal volumes of the two media, supplemented with 4 percent each of chicken and calf sera, supported growth to the same high numbers as the IPL–73 alone, with a population doubling time of slightly more than that of IPL–52 alone (82 hours).

Animal serum supplementation of culture medium for use in the production of the gypsy moth virus was considered basically undesirable for two reasons: The sera are possible sources of undesirable viruses and
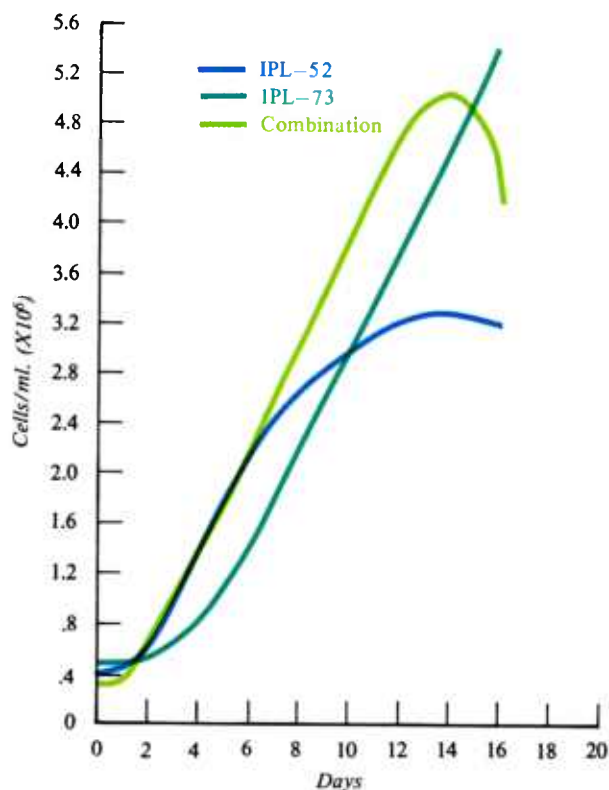
Figure 6.3–8.—*Growth of IPLB–LD–652 cells in the amino acid based medium, IPL–52; the peptone-based medium, IPL–73; and a combination of the two media. All media were supplemented with 3 percent each of turkey, chicken, and fetal bovine sera.*

mycoplasmas, and they are very expensive. Serum supplementation at the levels described here would approximately double the cost of the medium. Therefore, some studies on medium formulation have included the definition and inclusion of critical components that would reduce or eliminate the serum requirement.

A serum-free combination of equal volumes of the IPL–52 and the IPL–73 media was found to support slow serial passage of the IPLB–LD–652 cell line but no NPV replication (Goodwin 1976). Growth on this medium is shown in figure 6.3–9. Previous work with other cell lines indicated that insect cells required cholesterol and fatty acid supplementation (Vaughn 1973). Therefore, a lipid supplement containing cholesterol (1.5 mg per liter), methyl oleate (5.0 mg per liter), and Tween®-80 (25.0 mg per liter) in a filtered chloroform solvent was coated onto sterile glass beads and suspended by shaking in a sterile concentrated peptone fraction from the IPL–73 medium for

inclusion into the IPL–52–73 serum-free medium combination. This lipid supplementation resulted in improved cell growth over the same medium combination lacking the lipid supplement (fig. 6.3–9). Deletion of glycerol, while not greatly influencing cell growth in serum containing medium, resulted in growth failure in the third to eighth serial passage on serum-free, glycerol-free medium, depending upon the cell strain and passage number. This unusual glycerol requirement may indicate a failure in cellular membrane formation or an inadequacy in the lipid carrying capacity of the peptone concentrate used to bind the lipid supplements.

Virions formed in the lipid supplemented medium were enveloped but only rarely were occluded in polyhedra. Virus-challenged cells in this medium characteristically contained many intranuclear, free virons with a few empty polyhedra. This result indicates that specific serum-related factors that control the virus occlusion process are still absent from the serum-free medium.

The final delineation of the required critical serum factors may allow practical commercial-level poly-hedral formation in serum-free medium using the volume fermentation apparatus described in the following section.

Volume Cultures

Roller Bottles

Five strains (65CP, 65X, 652, 65W, and 65Y) of the IPLB–LD–65 line were tested in a roller-bottle culture system developed for culturing two cell lines from *S. frugiperda* (Vaughn 1976). Glass roller bottles were conditioned by adding 25 ml of growth medium to each bottle and rolling the bottles for 18 to 20 hours. Then the bottles were inoculated with $20 \times$ to 30×10^6 cells and rolled an additional 18 to 20 hours before the final volume was adjusted to 75 ml.

Although a fivefold to sixfold increase in cell numbers was obtained after 8 days, most of the cells were not attached to the surface but were floating in the medium. Plastic roller bottles, treated to permit cell attachment, did not provide any better
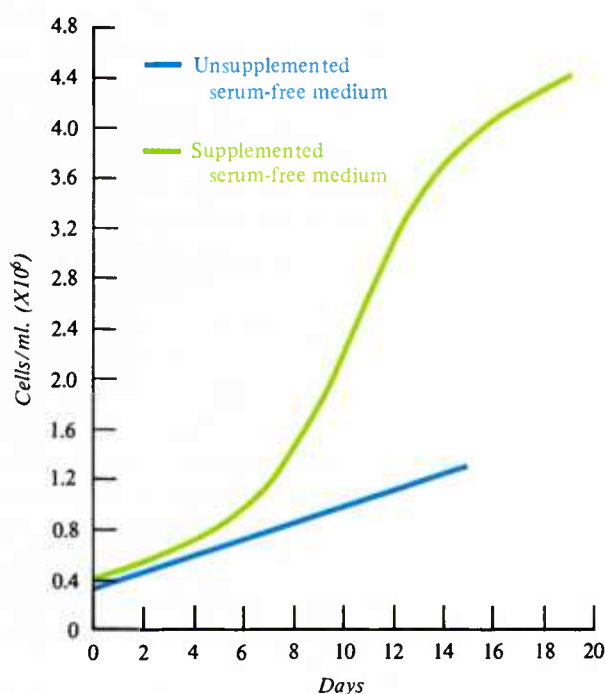
Figure 6.3–9.—*Growth of IPLB–LD–652 cells in serum-free medium, unsupplemented and supplemented with lipids.*

attachment than that obtained with glass. Without firm attachment it was not feasible to change media or otherwise manipulate the cultures, and it was not likely that the cultures could be further scaled up in size by using multisurface bottles. For these reasons, this system was abandoned, and the entire effort was devoted to various suspension culture systems.

Suspension Cultures

Insect cells, such as those of the IPLB–LD–65 line, that do not require attachment to a substrate as a condition for growth have been grown in various suspension culture systems (Vaughn 1968, Hink et al. 1974). The cells were maintained in the growth medium by slowly rotating bars or discs driven by external magnetic stirrers. Methyl cellulose or Darvan No. 2® (B. F. Goodrich Co.) was added to the standard growth medium to prevent damaging the cells by shearing and to prevent clumping. Cell yields and growth rates in such systems were generally equal to those of the various cell lines in static cultures. The two factors deemed to be of critical importance in the suspension cultures were adequate mixing, to provide optimum exchange of nutrients and metabolic by-products between the growing cells and the medium, and an adequate supply of oxygen.

These factors were examined in several culture systems beginning with 100-ml cultures in spinner flasks. To protect the cells from the effects of shearing, 0.25 percent methyl cellulose (400 centipoise) was added to the IPL–52 growth medium. When cultures were to be aerated, 0.2 ml per liter Antifoam B® (DowCorning Corp.) was also added to the medium. The cells were maintained in suspension by spinner speeds ranging from 100 to 425 rpm. At 425 rpm, the cells were severely damaged, so the maximum speed tested was limited to 300 rpm. The population doubling time, the average percent viable cells in the log phase, the net increase in viable cells, and the maximum cell numbers achieved were determined.

The results of this study are shown in table 6.3–11. All parameters tested gave significantly poorer results at a rotation speed of 100 rpm. It was thus concluded that this speed was too slow to provide adequate

Table 6.3-11.— *Effect of rotation speed on growth of* L. dispar *cells in 100-ml suspension cultures*

Rotation of stirring bar (rpm)	Population doubling time (hour)	Net increase in viable cells ($\times 10^6$)	Average percent viability	Maximum cell number per milliliter ($\times 10^6$)
100	76.14a[1]	2.10ac	87.58ab	2.74ac
150	72.97ab	3.21b	90.73a	3.66b
200	70.75b	2.17ac	86.10ac	3.11bc
300	73.89ab	2.63abc	79.78bc	3.34bc

[1] Means followed by the same letter are not significantly different at the 5 percent level, based on Duncan's Multiple Range Test.

mixing although the cells remained suspended. The slightly increased population doubling time and the low average percent viability at 300 rpm was thought to indicate some loss of cells due to shearing and thus represented a maximum speed level. A rotation speed of 200 rpm gave the best population doubling time. All other parameters showed maximum values at 150 rpm but with insignificant differences at 200 or 300 rpm. The single exception was at 200 rpm, where, unexplainably, significantly lower maximum cell number was observed. Thus a rotation speed of between 150 and 200 rpm was determined to be optimal. To test the suitability of these conditions for long-term propagation of the gypsy moth cells, two spinner cultures of cells from the IPLB–LD–652 line were established. The cell level was maintained between 500,000 and 3,000,000 cells per milliliter by the periodic removal of cells and spent medium and its replacement with an equal volume of fresh medium. One culture was maintained in logarithmic growth for 131 days with an average cell viability of 95 percent. The second culture was maintained for 50 days (average viability 86 percent) before it was used in another study. Such cultures established the suitability of the suspension culture system for growing cells of the gypsy moth. In a production system they could provide the initial seed inoculum for the larger production cultures.

The culture volume used in the suspension culture system was then increased to 250 ml in larger vessels of the same design. Similar tests were made, and the

optimum rotation speed was determined to be 250 rpm. In addition, the utility of the addition of oxygen was tested. For these tests, the standard spinner vessels were modified by replacing the solid glass rod to which the spin bar was attached with a glass tube and by connecting this tube to the air line. The cultures were aerated with humidified air that was sterilized by passage through Gelman bacterial air vents. The flow was controlled by needle valves, and the rate was determined by a Gilmont flowmeter having a range of 0 to 100 ml of air per minute. As shown in table 6.3-12, no improvement in growth was obtained in aerated cultures. Again, a long-term culture was established as before but with a volume of 250 ml. The culture was established from the 100-ml culture described earlier and continued for an additional 39 days until it was lost because of contamination. During the 39-day term, the average viability was maintained at 86 percent.

In similar tests with cultures of 500 ml, it was also determined that 250 rpm was the optimum rotation speed and that air was required for optimum growth. The data in table 6.3-13 show that without aeration growth stopped by the eighth day and that twice as many cells were obtained in aerated cultures as in those without air. Furthermore, in the aerated culture, growth continued for an additional 7 days; the final cell density was three times that of the nonaerated culture. The effect of air flow at various rates, ranging from 10–35 ml of air per minute, was studied, but no significant differences were found among these flow rates.

Table 6.3-12.—*Effect of aeration on the growth of* L. dispar *cells in 250-ml suspension cultures*

Maximum cell number ($\times 10^6$)	
Without air	With air
4.08	1.76
2.10	2.60
2.60	1.81
y=2.927[1]	y=2.057[1]

[1]Means were tested with Student's test and were not significantly different at the 1 percent level.

Table 6.3-13.—*Effect of aeration on growth and viability of* L. dispar *cells in 500-ml suspension cultures*[1]

	No aeration		Aeration	
Days	Cells per ml ($\times 10^6$)	Viability (percent)	Cells per ml ($\times 10^6$)	Viability (percent)
1	0.44	93	0.48	96
4	.88	95	.98	93
8	1.21	93	1.99	94
12	1.42	94	3.19	95
15	1.38	94	3.36	83

[1]Vaughn and Goodwin (1977).

As a final step in scaling up the gypsy moth suspension culture system, some tests were made with the Moduculture System (VirTis Company, Gardiner, N.Y.), a system that employs a spinfilter instead of a spinning bar to maintain the cells in suspension. The construction and geometry of the rotating filter are such that cells are maintained in suspension and medium can be withdrawn through the filter without removing cells or clogging the filter. Culture volumes of 2,500 ml were used in these vessels. A typical growth curve for the IPLB–LD–652 cells is shown in figure 6.3–10. By comparing this growth curve with those in figure 6.3–9, one can see that these cells grew as well in suspension culture as in static, flask cultures (pdt=84 hours). The growth represents a fivefold to sixfold increase in cell density over the starting inoculum. The yield is less than that obtained in static cultures but is sufficient to provide the cell inoculum necessary for the next step in a scale-up process.

Virus Production in Large-Scale Cultures

Although large volumes of cells could be produced, the virus yields were not sufficient to make commercial production feasible at this time. The PIB yields obtained from several different sublines of the IPLB–LD–65 line ranged from 5.2×10^6 to 3.4×10^7 PIB's per milliliter of culture. The highest yields, $3.0 \times$ to 3.4×10^7 PIB's per milliliter, were obtained with sublines 652 and 65X, respectively. Although this represented nearly an increase of 100-fold over some of the first yields, the average yield was only
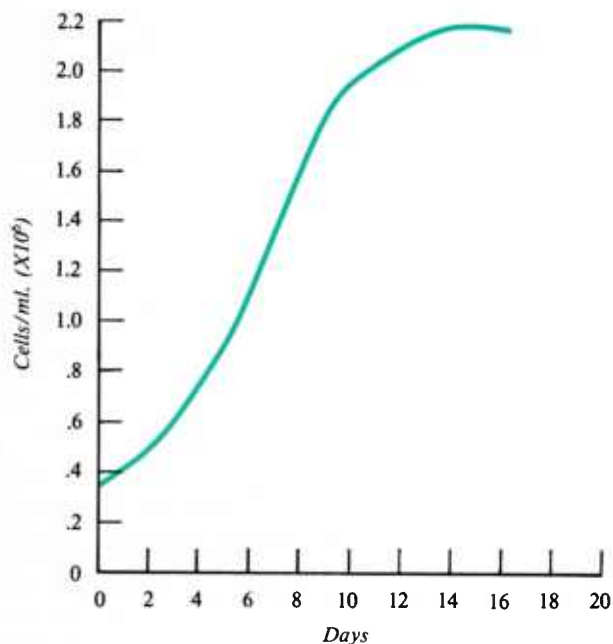
Figure 6.3–10.—*Growth of IPLB–LD–652 cells in IPL–52 medium in 2,500-ml suspension cultures.*

10 PIB's per cell, because cell densities of $2 \times$ to 5×10^6 cells per milliliter of culture were obtained.

Some factors that influence the yield of PIB's were identified during this study. Nutrition is one important factor. The peptone-supplemented, serum-free medium developed for the gypsy moth cells would support cell growth, but these cells produced few or no PIB's (Goodwin 1976). It was not determined whether this was because of the absence of certain nutrients or to an insufficient amount of the nutrients needed for both cell growth and virus replication. Oxygen demand was shown to increase for infected cells. As was described earlier the cells grew well in 250 ml spinner cultures without aeration. However, these cells were not capable of virus replication unless the culture was aerated. The physiological state of the cells also was found to affect virus production. Cultures inoculated with virus when the cells were in the stationary phase produced few or no PIB's. Only cultures of cells that were dividing would replicate the virus and produce polyhedra. Although all these factors were identified as influencing the production of PIB's, none was examined in sufficient detail to determine the conditions necessary for the maximum production of PIB's.

Summary

In reviewing the problem of producing viruses in cell cultures on a commercial scale, Ignoffo and Hink (1971) wrote: "The need must be coupled with major developments in insect tissue [culture] technology" and they listed four areas in which such developments were needed: "(1) development of prolific, high yield per volume cell lines; (2) simplification of culture media; (3) propagation of high pathogen titers in selected cell lines; and (4) design and development of plant-scale equipment and routine production procedures." During the course of this project, considerable development in at least three of these four areas was accomplished.

Cell lines that grow to high densities and that will replicate the gypsy moth NPV were developed and characterized. These cell lines have been tested for adventitious microorganisms and appear to be contaminant free. Methods for long-term storage in liquid nitrogen used for other cell lines were satisfactory for preserving the gypsy moth cell lines. Improvements in the culture medium used for these cells has permitted a reduction of levels of serum supplementation required to support complete viral replication. The serum-free medium supported adequate cell growth but not virus replication. Since sera represented about 50 percent of the cost of the complete medium, a further decrease in the serum requirements for virus formation would result in a considerable reduction in cost.

The success in growing gypsy moth cells in suspension culture indicated that the instrumentation and methodology for the large-scale production of animal cells could be used, possibly with some minor modifications, for the culture of insect cells. Improved procedures for handling the cells would increase efficiency and virus yield and decrease the cost of production. If procedures could be developed for producing synchronized cultures and determining the

most appropriate time in the cell cycle to infect them, yields would be further improved. Improvements in methods of supplying the proper levels of oxygen to the cells during virus replication are also needed to improve yield.

It is in the third area listed by Ignoffo and Hink (1971)—propagation of high pathogen titers—that the most research is still needed. Light-microscope observations have shown that some cells produce many more than 10 PIB's each, so there is a clear potential for a further increase in PIB production. The multiplicity of infection (MOI) has been demonstrated to affect the yield of *A. californica* virus obtained from *T. ni* cells (Brown and Faulkner 1975). Yields of PIB's were about 20 per cell when the *T. ni* cells were inoculated with a MOI of between 0.01 and 4.0. The yield tripled to 60 PIB's per cell when the MOI was increased to 20 or 30.

Cloning of either or both the cells and the virus may also increase the yield. If the variation in the numbers of PIB's per cell observed in these cultures is at least partly dependent upon the cell, then high-yield cells will have to be cloned and propagated to increase the efficiency of the production. Hink and Vail (1973) have shown that *A. californica* NPV isolated from insects contains at least two plaque variants. One variant produces few PIB's per cell and the other many. Since no plaquing system has been developed for *L. dispar* NPV, it is not known whether or not such variants occur, but it may be necessary to plaque-purify the virus inoculum before using it in production.

The results of this study have demonstrated that the production of gypsy moth NPV in tissue culture is technically possible. An increase in PIB yield of 50 to 100 times will be necessary to make the system economically feasible, but avenues of research that would make this increased yield possible have been determined and could be pursued further.

Safety Evaluations
Franklin B. Lewis and John D. Podgwaite

The knowledge of the safety of pesticidal materials is important for their proper use and is required for their registration. Materials are evaluated on their acute effects on mammalian species, subacute or long-term effects on mammals, and the effects on fish, wildlife, and beneficial organisms. Doses for testing the safety aspects of pesticidal materials are usually based on the weight of material per weight of test animal that causes 50 percent mortality. Test doses are usually multiples of the LD_{50} dose.

With the NPV's, including that of the gypsy moth, 50 percent mortality rates could not be determined because the material is essentially nontoxic or non-infectious for vertebrates. The lack of toxicity of NPV's led to the establishment of a test dose based on a multiple of a dose-per-acre applied to a 70-kg man prorated to the weight of the test animal. For example, the present dose rate of gypsy moth NPV is 1×10^{11} PIB's or 25×10^6 potency units per acre applied twice. Thus, if a 70-kg man received 2×10^{11}, a 20-g mouse would receive 5.7×10^7 PIB's. Test doses are usually used at $10 \times$ and $100 \times$ the field dose.

The NPV is administered in one massive dose for acute safety studies, while subacute doses are administered at intervals to add to the total dose over the length of the test (that is, 1/730th of the total dose daily for 2 years in the case of a 2-year feeding test). Test protocols follow accepted published guidelines or are developed in consultation with officials.

Since test procedures, guidelines, and developments have been and are constantly changing, it is advisable to consult with appropriate EPA officials prior to the conduct of specific tests. Some general guidelines have been published (Summers et al. 1975). Table 6.3–14 summarizes the range of tests conducted with gypsy moth NPV.

Actual Tests
Acute Oral

One 35-day and two 30-day tests were conductd on gypsy moth NPV products (Cannon Labs. 1976, Litton Bionetics 1975a, 1976.)

In test 6E–2618, 40 Sprague-Dawley rats were fed a single NPV dose equivalent to a 100-acre dose. Forty rats served as controls. The test, run for 35 days, was evaluated in terms of mortality, overt behavorial

Table 6.3–14.—*Summary of tests conducted with* L. dispar *NPV*

Test	Status Com-pleted	Status In progress	Effect
Acute, Mammalian:			
Oral	X		Nil
Eye irritation	X		Nil (slight)
Primary skin	X		Nil
Inhalation	X		Nil
Dermal	X		Nil
Immunodepressed	X		Nil
Acute, Fish:			
Trout	X		Nil
Bluegill	X		Nil
Acute, Wildlife:			
White-footed mouse	X		Nil
Shrew	X		Nil
Opossum	X		Nil
Quail	X		Nil
Mallard ducks	X		Nil
Acute, other Invertebrates:			
Daphnia	X		Nil
Notonecta	X		Nil
Chironomid	X		Nil
Honeybee	X		Nil
16 species Lepidoptera	X		Nil
Housefly	X		Nil
Subacute, Mammalian:			
Rats, 24 months	X		Nil
Dogs, 90 days	X		Nil
Serology:			
Identity	X		Some common antigen
Relation to arboviruses	X		No relationship
Human exposure	X		No antibodies
In vivo vrs. in vitro	X	X	Same
Biochemical-biophysical:			
Molecular weight	X		
DNA (homology)	X	X	
restriction endonuclease		X	
Environmental persistence:			
Leaves	X		Short life
Bark	X		
Litter	X		
Soil	X		
Sprayed and natural	X		Spray adds nothing
Tissue Culture:			
Vertebrates	X		
Invertebrates	X		
Other tests			
Product quality	X		
Product storage	X		

Test	Status Com-pleted	Status In progress	Effect
Product bioassay	X		
Product potency unit	X		
Primary standard	X		
Product production			
In vitro		X	LD_{50}'s
In vivo	X	X	Similar
Product formulation			
Compatability	X	X	
Simplicity	X	X	
Environmental resistance	X	X	
Product efficacy			
Ground	X		
Air	X	X	
Spot		X	

changes, body-weight gain, food consumption, body temperature, hematology, clinical chemistry, urinalysis, necropsy (including weight of seven organs), and histological examination of 19 tissues in each group. No abnormalities were found due to the administration of the gypsy moth NPV. Mortality was caused by *Corynebacterium kutscheri* in eight control and three test animals. This organism, a rodent pathogen, was not present in the test material and was considered to be carried by the test animals. Because of the involvement of the bacteria, the two additional tests were conducted. It was concluded that the NPV test at the 100 acre dose showed no effects on the test rats.

In addition to the toxicity testing in the 30-day test, sample tissues were bioassayed to determine if the NPV or an infectious breakdown product was accumulated in tissues. This test indicated that the NPV was either destroyed or eliminated from the gut within 72 hours. Ignoffo et al. (1971) demonstrated similar data for the *Heliothis* NPV.

Liver tissue showed some residual activity 14 days postingestion, but this is ascribed to contamination of the tissue during removal from the animal.

Acute Eye Irritation Tests

Acute eye tests were conducted by placing 50 mg of the NPV product Gypchek in the right eye of 12 New

Zealand albino rabbits. The left eye of each animal served as the control. The test was evaluated for 14 days. One group of rabbits had the treated eye washed 10 seconds after instillation, another group had the eye washed 1 minute after instillation, a third group's eyes were washed after 5 minutes, and the fourth group received no washing. Eye irritation did not occur in the quickly washed eye animals, but persisted in the unwashed and 5-minute exposure animals. Although the test dose was far above that expected to be encountered, the test results caused the material to be classed as a moderate eye irritant. Microbiological examination of the eyes showed no involvement of microorganisms; the irritation is apparently due to the insect hairs in the product.

Acute Inhalation Studies

Eighteen Sprague-Dawley rats were exposed for 1 hour to a concentrated dust of the gypsy moth NPV flowing at 12 l per minute. No signs of toxicity or abnormal behavior were noted in any rat during or following exposure, no animals died, and no treatment-related abnormalities were noted at necropsy. Bioassay of lung, bronchii, and trachea indicated rapid removal of infectious particles from the respiratory tract.

Primary Skin Irritation

Sixteen New Zealand albino rabbits were tested with intact and abraded skin to which 1 g of Gypchek was applied. Observations were conducted for 21 days. Gross pathologies and microscopic evaluations were made of 12 major organs. No death or abnormal behavior was observed. No abnormal pathologies were observed, and no dermal effects were noted (Cannon Labs. 1976).

Acute Studies on Immunodepressed Animals

A special study of immunodepressed animals was conducted under the direction of Dr. R. E. Shope at the Yale Arbovirus Research Unit (Shope and Tignor 1977). Mice and guinea pigs were immunodepressed by several methods and were challenged with purified polyhedra or free virus rods. Several routes of exposure were tested (oral, inoculation, inhalation, and eye instillation). Single-exposure tests and one 90-day feeding test were conducted in mice, and dermal toxicity tests were conducted on guinea pigs.

The general appearance and health of mice and guinea pigs exposed to *L. dispar* NPV with and without immunodepression were comparable to control animals given saline and to control animals given autoclaved NPV. Some mice inoculated in the footpad developed localized bacterial abcesses, but these were determined as secondary and not due to direct effects of the NPV.

Histopathological examination of the animals indicated there was no consistently detectable lesions in the mice or guinea pigs due to exposure to *L. dispar* NPV. One mouse died of pneumonia.

Subacute Studies

Dog Studies

Twenty-seven young adult purebred beagle dogs were fed gypsy moth NPV at three dose levels for 180 days (approximately 1-, 10-, and 100-acre equivalents). Each dog was observed daily for appearance, behavior, appetite, and elimination; toxic or pharmocologic effects were recorded daily. Hematology, clinical biochemistry, and urinalysis were performed during quarantine period and at the second, fourth, and sixth month after exposure. At the termination of the study, gross pathology was done, and histopathological examination was made on five selected organs. Twenty-four tissues were preserved for future histopathological determination.

Beagle dogs given the daily doses of the gypsy moth NPV showed no important changes in blood cytology or chemistry or urinalysis. Gross pathology examination revealed no important findings. Histopathological examination of tissues indicated no abnormal findings (Litton Bionetics 1975b).

Two-Year Rat-Feeding Study

Gypsy moth NPV was fed to albino rats over a period of 2 years. Total ingestion of PIB's equaled 10× and 100× the field equivalent dose. In each of the two dose schedules, 100 rats were used plus 100 control rats.

All animals were necropsied when found moribund or at the conclusion of the test. Seven organ weights were tabulated, and 31 tissues (from each animal) were preserved for histopathological examination. Treatment did not influence survival, weight, or food consumption. The tumor incidence or other microscopic lesions found were not attributable to the treatment (Litton Bionetics 1975c).

Beneficial Insect Testing

Honey bee, *Apis mellifera,* has been challenged with insect microbial agents, including gypsy moth NPV. Published reports (Cantwell et al. 1966, Cantwell et al. 1961, Knox 1970) indicate no deleterious effects attributed to the pathogen incorporated in sucrose solutions and fed to bee colonies for up to 4 months.

Aquatic Invertebrate Tests

Selected common aquatic invertebrates were exposed to high concentrations of gypsy moth NPV. The concentration used was approximately that which would occur when a very shallow pond was sprayed at the rate of 3.75×10^{13} PIB's per hectare.

The invertebrate species tested were the plantivore *Daphnia magna; Notonecta undulata,* a predator of *Daphnia;* a benthic invertebrate, *Chironomus thummi;* and two species of water boatmen that feed by scraping algae and other associated material from submerged aquatic surfaces.

Survival of *Daphnia, Chironomus,* and *Notonecta* was unaffected by exposure to the NPV during their development from first instar to adult. Further, the development time of the immatures and subsequent reproduction of the treated adults were comparable to control insects.

Bioassay of specimens from these tests indicated that *Daphnia, Notonecta,* and water boatmen did not accumulate gypsy moth NPV (Streams 1977).

Effect of NPV on Apanteles melanoscelus

Ten mated *Apanteles melanoscelus* females were fed *L. dispar* NPV for 7 days at the rate of 2.8×10^8 PIB's per milliliter in honey water. A similar group was fed for the same time on plain honey water.

These wasps were then exposed to newly molted second-instar larvae in groups of 20 for a 4-hour period for 5 consecutive days.

Longevity of the wasps, percent parasitization, and sex ratio of emerging next-generation wasps were not significantly different between treatment and control.

Susceptibility of Insects to Gypsy Moth NPV

Ten Lepidopterous, two Hymenopterous, one Coleopterous, one Orthopterous, and one Dipterous species were challenged with *L. dispar* NPV at a dose of 1.5×10^8 PIB's per milliliter, an extremely high dose. Despite the high doses, no apparent effect of these treatments was noted. Cross transmission of NPV has been reviewed by Aizawa (1963). Weiser and Verber (1954) reported no effect when *L. dispar* NPV was tested against *H. cunea,* and Smith (1967) reported *L. monacha* NPV noninfectious for *L. dispar.* Martignoni (1977) indicated that mortality of *O. pseudotsugata* larvae occurred when fed *L. dispar* NPV.

Fish and Wildlife Tests

Fish

Ninety-six hour static exposure tests were conducted with 240 juvenile bluegills and 240 juvenile brown trout.

As a result of this study, which examined the effects of gypsy moth NPV on survival and histopathology of bluegills and brown trout, it was concluded that the NPV had no demonstrable effect on either species at doses of approximately 100 times the field application dose. (Moore 1977).

Game Birds

Bobwhite quail and mallard ducks were challenged with 100 times the field dose of gypsy moth NPV. No effect was apparent in either species with regard to toxicity, behavior, or mortality due to the oral administration of the gypsy moth NPV.

Effects on Natural Vertebrate Predators

NPV was fed to the white-footed mouse, *Peromyscus leucopus;* the shorttailed shrew, *Blarina brevicauda;* and the Virginia opossum, *Didelphis*

marsupialis, as both purified PIB's and NPV-infected gypsy moth larvae (Lautenschlager et al. 1977). Results of this study indicated that the ingestion of NPV at doses each equivalent to more than a 40-ha exposure for a 70-kg person had no short-term effects on these important predators of the gypsy moth.

NPV was fed to the black-capped chickadee, *Parus atricapillus,* and to the house sparrow, *Passer domesticus,* as NPV-infected gypsy moth larvae (Podgwaite and Galipeau 1978). Analyses of body weights and histopathological examination of organs from NPV-treated birds indicated that NPV had no apparent short-term effects on these two avian predators of the insect.

Field studies were conducted to determine any adverse effects on wildlife following aerial applications of NPV to woodland plots in Pennsylvania (Lautenschlager et al. 1978, 1979). Resident small mammal and songbird populations as well as caged white-footed mice, opossums, and quail were evaluated after exposure to NPV applied at the rate of 2.5×10^{12} PIB's per ha. Comparisons of prespray and postspray censuses of the dominant mammals and common songbirds on control and NPV-treated plots indicated that no population changes could be attributed to NPV treatments. Further, necropsy and histopathological data taken on mammals and birds either free-living or caged in the study plots indicated that no significant differences existed between control and treated birds that could be attributed to NPV applications.

These studies have shown that gypsy moth NPV has no apparent adverse effects on those birds and mammals that may utilize the gypsy moth as a food source, or on those birds and mammals that may contact the virus from NPV spray, spray residue, or NPV-infected larvae.

Concluding Note

The tests summarized in this section were accomplished over a long period of time, starting in 1972 and ending in 1977. All the tests reported here were performed by contract with commercial firms or under agreements with universities or Federal agencies. The total cost for securing this safety data was approximately $300,000. The full text of all reports was submitted to EPA and is on file at the Hamden laboratory of the Northeastern Forest Experiment Station.

Environmental Persistence of Gypsy Moth NPV

John D. Podgwaite

Introduction

The knowledge of how long, where, and in what quantities NPV persists in the environment of its host is important for several reasons. First, it is the basis for understanding the processes by which NPV is spread both within natural populations of the insect and from one generation of the insect to the next. These processes may be modeled and used to predict population trends. Second, the efficient use of NPV as a microbial insecticide is based on, among other things, the length of time the virus persists in a viable state on foliage or on other environmental surfaces after its application. Finally, from the standpoint of safety, it is desirable to be judicious when adding NPV to the enviroment so as not to unnecessarily increase the risk of exposure of nontarget organisms to the virus. Thus monitoring the natural virus load and NPV spray residues is essential to the safe utilization of this virus in an integrated pest management program.

Location, Accumulation, and Persistence in the Environment

Although NPV has existed in North American gypsy moth populations since the early 1900's (Glaser and Chapman 1913, Reiff 1911), few studies have dealt with the location of this virus in the host's environment. Early work by Bergold (1958) revealed an association between the virus and the egg of the host, an association later shown to be important in the generation-to-generation transmission of the virus (Doane 1969). More recently, Doane (1975) showed that NPV persisted in insect debris collected from areas on trees where larvae of the previous generation had either congregated or pupated. He showed that larvae became infected with NPV, after hatching from disinfected eggs, by crawling through layers of this

debris. A laboratory study by Lautenschlager and Podgwaite (1977) indicated that viable NPV can persist in the feces of small mammalian predators of the gypsy moth. Further studies by these workers (Lautenschlager and Podgwaite 1979) showed that birds and large mammals also pass infectious NPV through their alimentary tracts after being fed either purified NPV or NPV-infected larvae. Thus it is likely that NPV persists in the excrement of these animals in the natural environment of the gypsy moth.

One of the major objectives of the expanded program was to register NPV as a microbial pesticide. Prior to the program, there was little research directed toward quantifying NPV levels in the environment, for two reasons. First, NPV has been a natural component of North American gypsy moth infested forests for 75 years or more and is produced in quantities that, at least theoretically, exceed by at least 1,000-fold those that would be added to the environment. Second, studies on other insect viruses (Jaques 1975, 1977) indicated that these viruses are rapidly inactivated by sunlight following their application and therefore have a low probability of contact with nontarget wildlife. These arguments notwithstanding, the Environmental Protection Agency (EPA) has taken a cautious approach to the registration of biological agents and has required potential registrants to bring forward information relating to the accumulation, persistence, and stability of these agents in treated areas.

Pursuant to EPA requirements, Podgwaite et al. (1979) conducted a study to determine how long, where, and in what amounts gypsy moth NPV persists after natural epizootics and how introduction of the virus affects its natural accumulation and persistence in the environment. A bioassay technique was used to estimate naturally occurring levels of infectious NPV in leaf, bark, litter, and soil samples taken from woodland plots in Pennsylvania and Connecticut. These levels were compared to those occuring in samples taken sequentially after treatment of these plots with NPV. The results reaffirmed that NPV is a natural component of the host's habitat and showed that it persists naturally at high levels in soil, litter, and on bark for at least 1 year following natural epizootics

(table 6.3-15). NPV levels in a treated plot are also shown in this table.

It was found that the NPV activity in spray deposits on foliage (fig. 6.3-11) was measurable for only 3-15 days after NPV treatment, whereas activity of NPV liberated from larval cadavers onto bark was measurable at high levels through the spring of the year following treatment. The loss of activity of NPV in spray deposits was believed to be due either to solar irradiation or to the physical removal of NPV from foliage and bark following heavy rains. It was also found that NPV contained in exudates from larval cadavers was more resistant to deactiviation than NPV in spray formulations. It seems likely that proteinaceous degradation products in the cadaver serve to protect NPV from harsh environmental factors and it is also probable that the rough surfaces and corrrugated nature of certain tree barks (fig. 6.3-12), particularly the oaks, afford additional protection of NPV from solar irradiation. Results also showed that NPV persisted at the highest levels, both naturally and after introduction, in the litter and in the soil. This was expected based on what is known of the accumulation and persistence of other NPV's (Jaques 1975, 1977). In soil, NPV is afforded maximum protection from sunlight and other adverse conditions that would result in desiccation or freeze-thaw fracturing of its PIB's.

This study also gave some indication as to how the introduction of NPV into the environment affects the natural NPV load. The application of NPV at the rate of 2.5×10^{12} PIB's per hectare to an area (Connecticut plot) already supporting high levels of naturally occurring NPV did not cause an increase in the environmental NPV levels. In fact, there was a 74-percent decrease in PIB's in the litter and a 57-percent decrease in PIB's in soil 1 year after treatment. In Pennsylvania, the application of NPV at the rate of 5×10^{12} PIB's per hectare to plots supporting low, natural levels of NPV resulted in measureable increases in the NPV levels in these plots. However, these increases were not significantly higher than those occurring in a control plot that was not treated with NPV (table 6.3-16). In fact, only NPV levels in soil were higher than those in the control plot.

Table 6.3–15.—*Estimation of the number of infectious PIB's of gypsy moth NPV on bark, in litter, and in soil in an NPV-treated and untreated woodland plot in Pennsylvania*

Sampling date	Bark[1]		Litter[2]		Soil[2]	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
May 1975[3]	9.63×10^3	3.65×10^3	9.80×10^3	1.07×10^4	1.38×10^4	1.65×10^4
June	8.80×10^5	1.79×10^5	3.47×10^5	1.33×10^5	4.49×10^5	9.35×10^4
July	7.90×10^6	7.03×10^6	9.61×10^5	7.39×10^5	4.55×10^5	3.21×10^5
August	8.33×10^6	3.24×10^6	5.39×10^6	7.19×10^5	4.30×10^5	2.42×10^5
September	1.64×10^6	8.47×10^5	9.61×10^5	5.31×10^5	3.18×10^5	4.52×10^5
October	2.94×10^6	6.76×10^6	6.46×10^5	8.15×10^5	2.99×10^5	4.47×10^5
January 1976	3.71×10^5	2.42×10^5	2.56×10^5	4.23×10^5	1.52×10^5	1.19×10^5
May	1.81×10^4	1.27×10^5	1.07×10^5	1.82×10^5	2.41×10^5	1.77×10^5

[1]PIB's per cm[2] based on four samples per plot
[2]PIB's per cc based on six samples per plot.
[3]May 1975 samples were taken prior to treatment with 5.0×10^{12} PIB's per hectare.
 Source: Podgwaite et al. 1979.

Summary

Data gathered to date indicate that gypsy moth NPV is a natural component of the host's environment and that it persists for extended periods. Further, the addition of NPV to the environment at levels consistent with those used for control of the insect does not raise NPV levels above those that would occur naturally. Hence it appears that there is only minimal risk of increased exposure to nontarget species when NPV is utilized as a microbial control agent. Finally, NPV persistence data have provided a foundation on which future NPV epidemiology studies can be based. Results of these studies will provide useful models describing the natural NPV disease system. Such models will be useful in making gypsy moth management decisions.
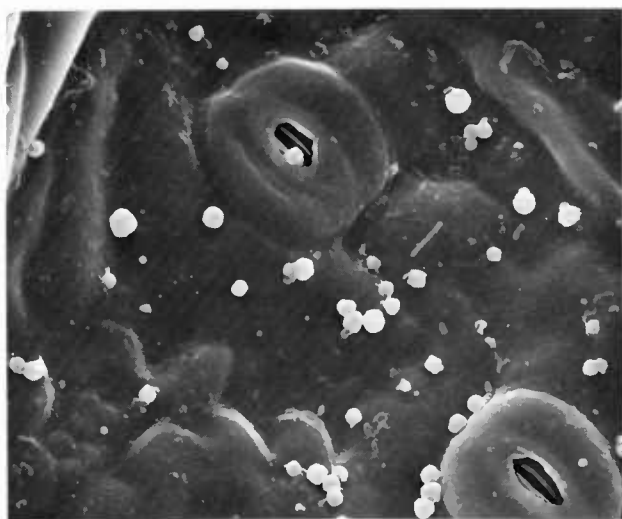
Figure 6.3–11.—*PIB's of gypsy moth NPV on leaf surface following application* (×1,200).
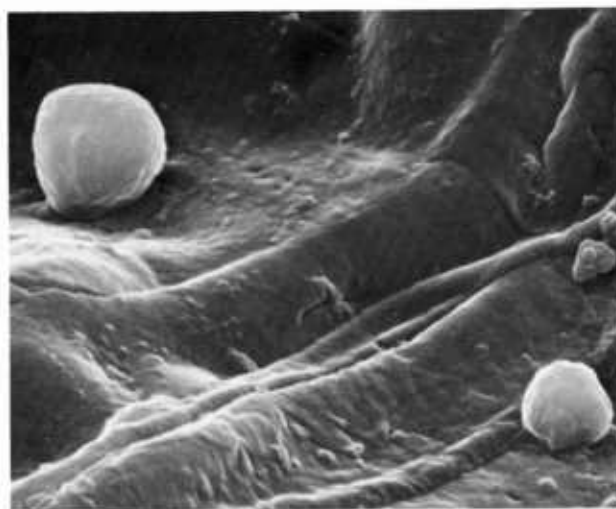
Figure 6.3–12.—*PIB's of gypsy moth NPV (upper left and lower right) on the surface of red oak bark* (×7,500).

Table 6.3-16.—*Changes[1] in NPV levels in two NPV-treated[2] plots and one untreated plot in Pennsylvania 1 year after treatment*

Strata	NPV-treated plots		Untreated plot
	1	2	
Bark	6.1	1.9	34.8
Litter	8.5	10.9	17.0
Soil	18.0	17.5	10.7

[1]Expressed as the number of times the prespray PIB level.
[2]Plots treated at 5.0×10^{12} PIB's per hectare.
Source: Podgwaite et al. 1979.

Epidemiology

John D. Podgwaite, Kathleen S. Shields, and Richard A. Lautenschlager

Introduction

Understanding how NPV is transmitted within and between generations of the gypsy moth is a complex problem, but one with a solution that could allow us to predict when NPV epizootics will occur and further allow pest managers to make intelligent control decisions. There are several mechanisms of NPV transmission: Physical factors such as wind and rain, mammalian and avian predators, entomophagous parasites, and the gypsy moth itself. How these factors integrate to bring about the dramatic expression of disease is still not well understood, but studies undertaken during the gypsy moth program have brought the answer closer.

Transmission by Avian and Mammalian Predators

The ability of birds to pass and translocate viruses of insects has been well documented (Bird 1955, Hostetter and Biever 1970, Entwhistle et al. 1977, *a, b*). Recently, this same ability has been demonstrated in two mammalian predators of the gypsy moth (Lautenschlager and Podgwaite 1977). Recognizing that both birds and mammals could be important in

the movement of NPV by circulating it within their immediate environment as well as by transporting it away from NPV-contaminated areas, Lautenschlager and Podgwaite (1979) tested several avian and mammalian species to determine: Which species pass significant amounts of active NPV; the percent of NPV ingested that passed and remained active; the passage rate of NPV, or the length of time after ingestion of NPV until all NPV was eliminated from the alimentary tract; and if passage rate is related to loss of NPV activity. A knowledge of these points is essential for determining the potential of any vertebrate species or vertebrate class to move significant quantities of this virus in the wild.

In these experiments, mammals tested ranged in size from the small white-footed mouse (*Peromyscus leucopus,* Rafinesque) and the short-tailed shrew (*Blarina brevicauda* Say) to the large opossum (*Didelphis marsupialis* L.) and raccoon (*Procyon lotor,* L.). Birds tested ranged from small house finches (*Carpodacus mexicanus* Muller) to large mourning doves (*Zenaidura macroura* L.). Gypsy moth NPV was fed to the test animals as known amounts of PIB's placed directly in their stomachs and NPV-infected gypsy moth larvae.

Determining the amount of NPV that passed through these species was achieved by counting PIB's in fecal suspensions and bioassaying these suspensions against gypsy moth larvae. Results of these tests demonstrated that all species passed NPV in amounts great enought to kill some gypsy moth larvae. The large mammals (opossums and raccoons) passed from 0.17 to 15.24 percent of the PIB's that they received— a mean of about 5 percent. This mean was about twice the mean passed by the small mammals (mice and shrews), which passed between 0.42 and 4.57 percent. Small mammals passed all NPV within 18 hours (the majority within 12 hours), while raccoons passed it within 22 hours and opossums within 70 hours. The smallest birds (house finches) passed the most NPV, between 2.21 and 33.76 percent, while the largest birds (mourning doves) passed the least—0.04 to 0.08 percent. The small birds passed PIB's more rapidly (within 2 hours) than the larger birds, which passed

PIB's within 6 hours. In conjuction with these experiments, a recent examination of field data determined that roughly 70 percent of the alimentary tracts taken from 109 mammals and 13 birds collected from areas experiencing NPV epizootics contained enough NPV to cause significant gypsy moth mortality in bioassays (Watson et al. 1978).

It is now obvious that both birds and mammals have the ability to pass and disperse active gypsy moth NPV. The amount dispersed, however, is dependent on the amount consumed; other foods available and consumed; and, in certain cases, the size of the animals involved. It is only by chance that areas are inoculated with NPV passed by vertebrates; however, clearly both birds and mammals will affect the movement of gypsy moth NPV within the environment, and some thought should be given to incorporating this potential into a program of biological control.

Transmission by Entomophagous Parasites

Casual field observations by many gypsy moth researchers over a number of years have indicated that entomophagous parasites may play a role in the transmission of disease agents within natural populations of the insect. Reardon and Podgwaite (1976) compared the incidence of NPV with the incidence of several parasites within several gypsy moth populations over a 2-year period. They found significant positive correlations between the incidence of NPV disease and the incidences of the parasites *Apanteles melanocelus* and *Parasetigena agilis* (=silvestris). A followup study by Raimo et al. (1977) showed that, indeed, *Apanteles* females could transmit NPV from infected to healthy gypsy moth larvae. Although this type of NPV transmission undoubtedly occurs in natural gypsy moth populations, to what extent it occurs and its importance in NPV epidemiology remain to be determined.

The results of the studies just mentioned have led to exploring the use of NPV-contaminated *Apanteles* to initiate NPV epizootics in natural gypsy moth populations. This is discussed in greater detail in chapter 6.1.

Generation-to-Generation Transmission of NPV

The gypsy moth may be a prime contributor to its own downfall. It has long been suspected that gypsy moth NPV is transmitted from generation to generation via the eggs. Bergold (1942, 1943) concluded that NPV was present on the surface of eggs because without disinfecting the egg surface few larvae ever lived to pupate. He also found particles, believed to be the PIB's, in the trichloracetic acid with which he washed the eggs. Doane (1969) found that surface disinfection of one population of eggs reduced the incidence of nucleopolyhedrosis in the larvae from 80 percent to 0.1 percent and concluded that transovum transmission of NPV takes place via the egg surface, resulting in early death of virus-susceptible larvae. Larvae from eggs collected at other times or from other sites experienced less than 10 percent NPV and were considered to be virus resistant.

Because these studies showed that surface disinfection of eggs appeared to reduce virus disease incidence, it was assumed that the mode of virus transmission was transovum; however, NPV was neither recovered from the surface of eggs nor identified as the causal agent. The possibility of transovarial transmission of NPV from an infected adult to the egg within the ovary was not investigated. Therefore, a study was initiated within the gypsy moth program to determine to what extent and in what way gypsy moth NPV is transmitted from one generation to the next. This study is still in progress but some general comments can be made on information thus far obtained.

Although evidence exists for surface contamination of eggs with NPV, the scanning electron microscope has thus far revealed only an occasional PIB on field-collected eggs. When eggs were hatched and larvae reared in the laboratory, some groups experienced up to 100 percent polyhedrosis, but PIB's could not be detected on the egg surfaces. Eggs, however, are frequently contaminated with various particulates, including spores that at low magnifications could easily be mistaken for PIB's (fig. 6.3-13). Figure 6.3-14 shows the same egg surface at a much higher

magnification and reveals characteristic morphology of spores as opposed to PIB's (fig. 6.3-15). Work is continuing to determine if some viral component other than the intact PIB is involved. This seems theoretically possible since surface sterilized eggs which were artificially contaminated with PIB's, purified virions, mixtures of lysed PIB's, or mixtures of virions and polyhedral protein all resulted in very high levels of nucleopolyhedrosis in the larvae.

Laboratory studies are being conducted to determine if NPV can be passed from one generation to the next by larvae with a chronic NPV infection or by larvae that have consumed large amounts of NPV shortly before pupation. The possibility of intro-ducing NPV into a population is also being investigated by testing the effect of artificial contamination of moths and egg masses under field conditions. This information will not only allow better understanding of NPV epidemiology but also permit more effective manipulation of this natural disease agent. In discussing NPV epidemiology, those mechanisms that ensure the survival of the virus from one generation of the insect to the next must be distinguished from those involved in the initiation of NPV epizootics. Although these mechanisms may not be mutually exclusive, they may differ, depending upon environmental factors and factors relating to the susceptibility of the insect to the disease.
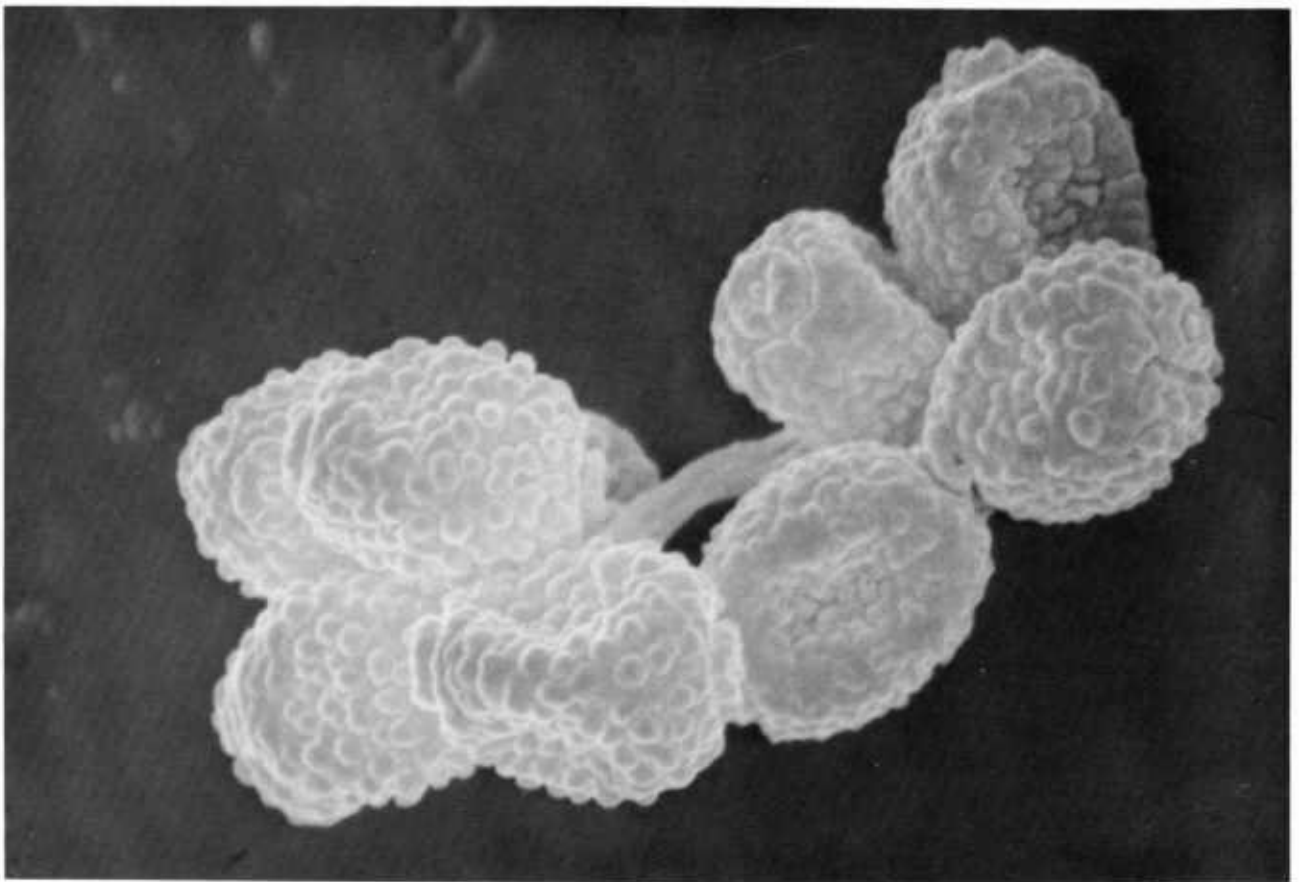
Figure 6.3-13.—*Surface of gypsy moth egg showing presence of unidentified particulates.*

As discussed earlier, the gypsy moth egg appears to be the major vehicle for transporting NPV from generation to generation. This concept is based upon much experimental evidence (Doane 1969, 1970). However, recent studies by Doane (1975) and by Podgwaite et al. (1979) have shown that gypsy moth NPV survives at high levels on environmental surfaces within gypsy moth populations and from one generation of the insect to the next. There are further data (Podgwaite 1978) showing that in the laboratory gypsy moth larvae can contaminate their food after having come in contact with NPV-contaminated bark surfaces. If this mechanism exists in natural gypsy moth populations, it could, as could the concept of viral latency, account for some of the inadequacies in the current theory of how NPV epizootics are initiated.

The current theory that is generally accepted by most researchers involves the following sequence of events: The NPV diseased female moth contaminates her eggs, either externally or internally, with NPV. Larvae hatching in the succeeding generation are either already infected or become so after chewing their way out of the contaminated egg. These infected larvae die in the first instar and become the source of NPV inoculum for the survivors. Surviving larvae of all stages contract the disease by feeding on NPV-contaminated foliage. Some female larvae that ingest
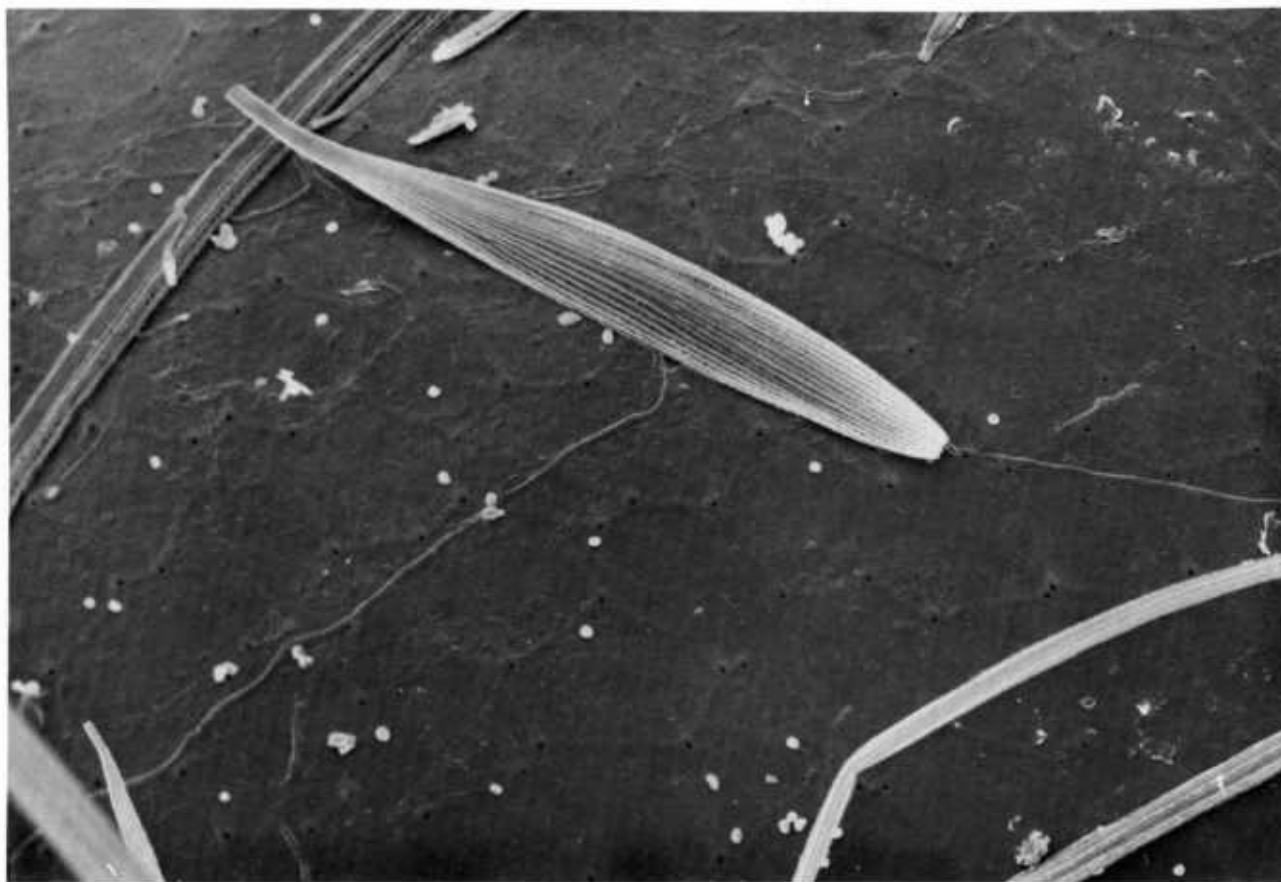
Figure 6.3-14.—*Higher magnification of egg surface shows particulates are spores* (×14,500).

PIB's and that are close to pupation do not die of the disease, but rather, through some mechanism as yet not clearly defined, transmit NPV to their eggs. A testable model based on this sequence of events is presented in chapter 8.

Whether or not this sequence of events actually represents what takes place in natural populations remains largely conjecture, since there are little field data either to support directly this theory or to refute it.

The question of whether NPV is on the surface of the egg or within the embryo is critical to assessing the validity of the current theory. If NPV is on the egg surface, this leads to the inevitable conclusion that

dead first- or second-instar larvae are the source of PIB inoculum for the initiation of epizootics. However, there is no direct evidence to show that, in fact, these dead larvae are the cause of epizootics in natural gypsy moth populations. If NPV is in the embryo as "latent" virus (of which there is also no direct evidence), this could account for the initiation of disease at any larval stage, in response to predisposing environmental factors.

Environmental factors such as temperature, humidity, wind, and rain may contribute to the initiation of NPV epizootics. Wallis (1957) suggested that at least high humidity may be linked to the initiation of polyhedrosis in gypsy moth larvae.
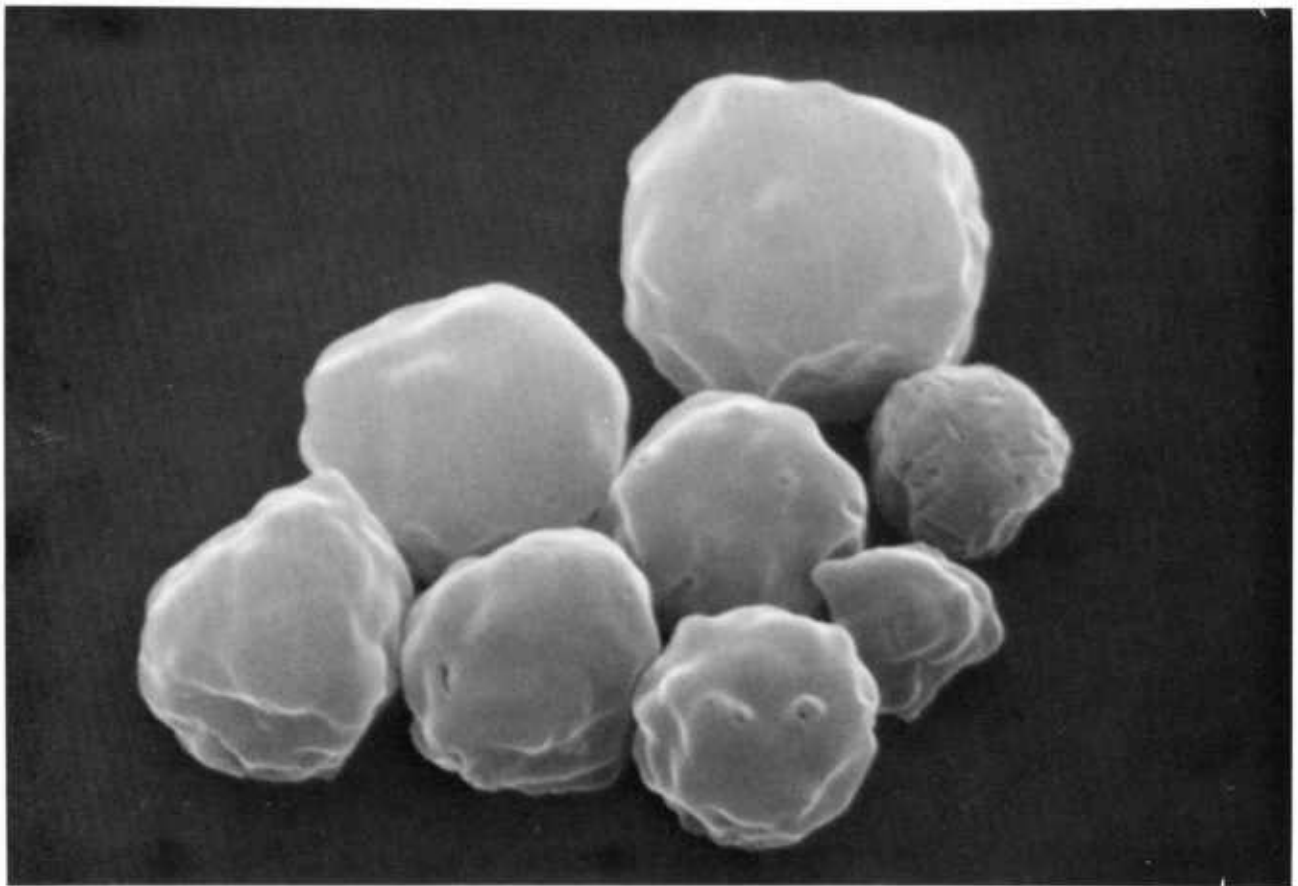
Figure 6.3-15.—*Typical PIB's of gypsy moth NPV* (×*17,000*).

Unfortunately, there is nothing, other than perhaps casual field observations, to link any environmental factor to the initiation of epizootics.

Entomophagous parasites or avian and mammalian predators of the insect may play a role in either the initiation or prolongation of NPV disease in natural populations. As discussed previously, their potential to transmit or translocate NPV has been demonstrated; however, no data exist that actually show their contribution in natural situations.

Finally, what of the role of the insect itself—its behavior, nutritional requirements, genetic potential, and general state of health—in its susceptibility to NPV? Other than an apparent correlation between some food plants and the incidence of NPV, very little is known.

Undoubtedly, understanding NPV epidemiology involves a far greater knowledge of the interaction of many physical and biological factors than now is available. Future studies should focus on the relative importance of these factors and how they integrate in the initiation of NPV epizootics.

Biochemistry and Biophysics
Horace M. Mazzone and William J. McCarthy

Introduction

Determination of the biochemical and biophysical properties of a virus serves to describe and distinguish it from other viruses. Gypsy moth NPV has such properties—the polyhedral inclusion body (PIB) and its proteins, and the virions consisting of protein and nucleic acid that allow investigators to distinguish them from those of other viral agents. These identifying characteristics are important in a biological control program because the virus can be monitored after it has been disseminated in the field for control purposes; in the production of the virus by in vivo or in vitro procedures, changes in the properties of the virus can be recognized; and stored virus material can be checked for any changes occurring in the structure-function relationships between the virus and its associated forms.

Properties of Polyhedral Inclusion Bodies

Larvae of gypsy moths infected with NPV become factories that produced virus particles; in the infection process, the metabolism of the cell is directed by the virus to produce more virus. The material used to form more virus comes from the cell itself, and the process results in death of the cell. The virus particles formed become surrounded by or included within the many-sided structure, the PIB (fig. 6.3–16).

The site in the cell where PIB's are formed is the nucleus (fig. 6.3–17). As more PIB's are formed, the nuclear membrane and later the cell membrane burst (fig. 6.3–18), spilling out PIB's into the body cavity of the insect. The skin of the infected larva takes on a shiny appearance, and if it is pierced, a whitish fluid containing millions of PIB's flows out. These events of the infection process occur in the larval stage, but only poorly or not at all in the pupal and adult stages. Larvae that survive the infection are capable of passing into the later stages with the possibility of viable eggs being produced.

PIB's vary in size, ranging from 1 to 10 μm (fig. 6.3–16), and, exclusive of the virus particles contained within its structure, consist chiefly of protein. PIB's stored in the dried state have been kept for decades and maintained the viability of the virus particles contained within (Bergold 1953). A major function of the PIB, therefore, is to protect the viral particles it encloses from physical, chemical, and biological agents. Although PIB's are remarkable structures in their ability to withstand breakdown by the action of organic solvents such as ether, acetone, and alcohol, they are affected by acids and alkalis. PIB's were observed by early investigators using the light microscope to change their appearance in the presence of acids and alkalis. Bergold (1947) made use of this observation and used a weak alkali solution to dissolve the inclusion bodies. He was able to show by means of transmission electron microscopy that alkali-treated PIB's contained virus particles.

PIB's of gypsy moth NPV can also be dissolved by treating them at room temperature with an alkaline solution of pH 10.5. In a few minutes the protein

structure of a PIB begins to break down to smaller size proteins. The rods contained within become apparent when viewed with an electron microscope (fig. 6.3–19). If the reaction is stopped at this time by decreasing the pH to about 8.5 with a weak acid solution, one is able to count the rods contained within a number of partially lysed inclusion bodies. Generally, there is a direct relationship between the size (diameter) of PIB's and the number of rods contained within. If the alkaline dissolution of the inclusion bodies is allowed to continue for about an hour at pH 10.5, most of the inclusion bodies break

down completely, liberating the virus rods (fig. 6.3–20).

The density of PIB's is 1.272, and this property has been used in a procedure (Mazzone et al. 1971) for the purification of large quantities of PIB's of gypsy moth NPV (Breillatt et al. 1972). Infected larvae are processed by homogenization and filtration procedures to eliminate all particles and debris. However, bacteria and hairs are still present, the latter being particularly allergenic to mammals, including man. The crude suspension is then layered over a sucrose gradient column and centrifuged. The PIB's band at their
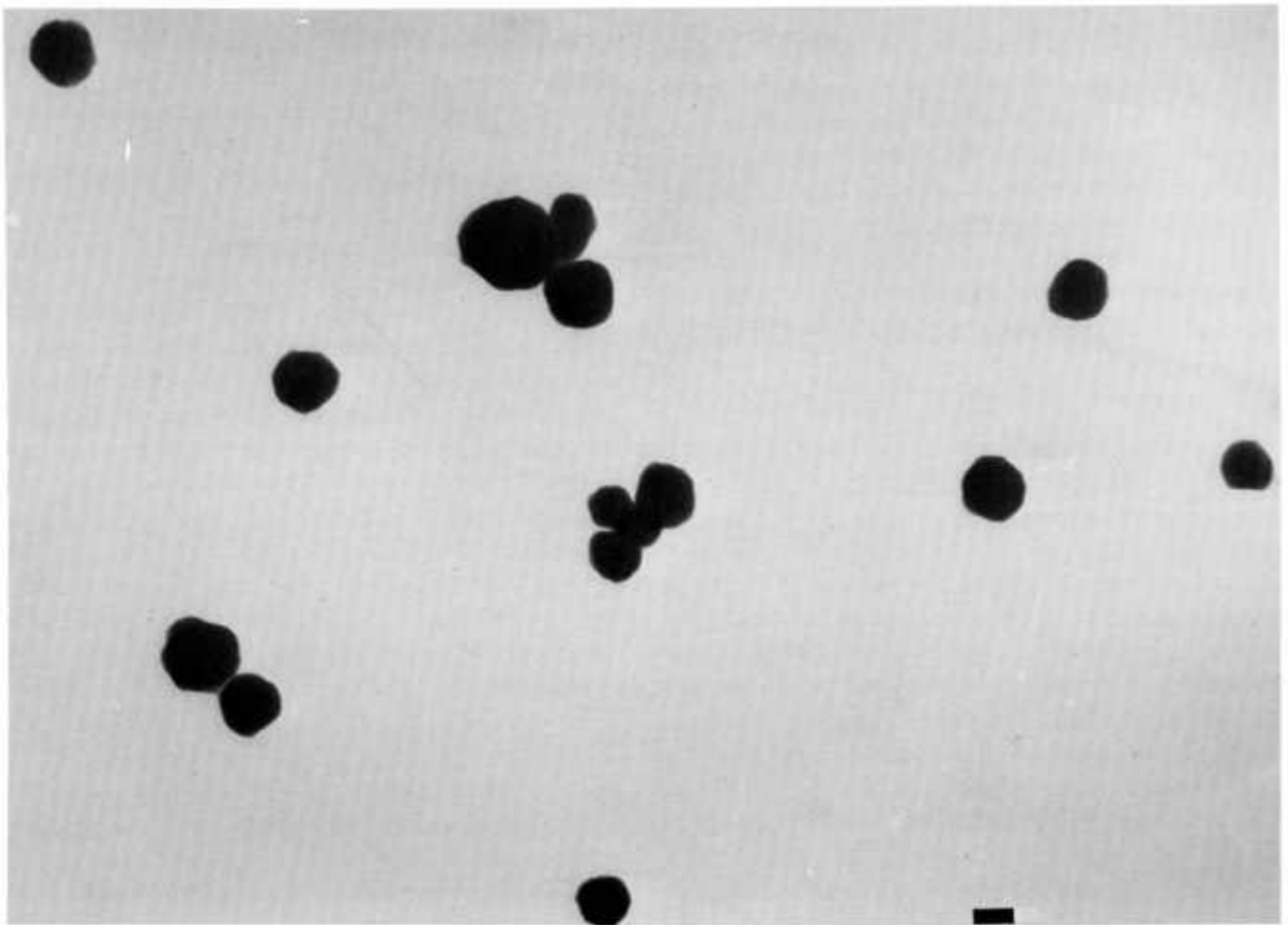
Figure 6.3–16.—*Polyhedron inclusion bodies of the gypsy moth nucleopolyhedrosis virus. The virus particles are contained within as shown in fig. 6.3–19. (Bar=1 μm.)*

density level and are recovered relatively free of contaminating substances.

PIB's have an average mass of 3.66×10^{-12} g. To weigh such particles, a transmission electron microscope method was employed (Bahr et al. 1976) in which the value of the transmitted electron beam through the particle was compared to that through a standard particle of polystyrene latex. Because of the dense packing of PIB's in the gypsy moth, it became necessary to employ the 1 million volt transmission electron microscope. The electron beam generated by this electron microscope was adedquate to penetrate the PIB's and transmission values were obtained for over 200 PIB's. Since the transmission value and mass value were known for the standard particles, the mass values for the PIB's were obtained by proportionalization, and an average mass value calculated. This value is of importance in field applications involving the spraying of formulations containing PIB's. The approximate amount of PIB's in a formulation is the product of the number of PIB's present (readily obtained by light microscopy) and the average mass value.

Figure 6.3–18.—*Two blood cells from a virus infected larva of the gypsy moth. The PIB's, which originated in the nuclei, increased to such numbers that they burst through the cell membranes.* (*Bar= 10 μm.*)

Figure 6.3–17.—*Larval blood cells* (*hemocytes*) *showing effect of viral infection. The nuclei of the cells contains PIB's.* (*Bar= 10 μm.*)

Figure 6.3–19.—*Partially lysed PIB revealing virus particles* (*rods*). (*Bar= 1 μm.*)
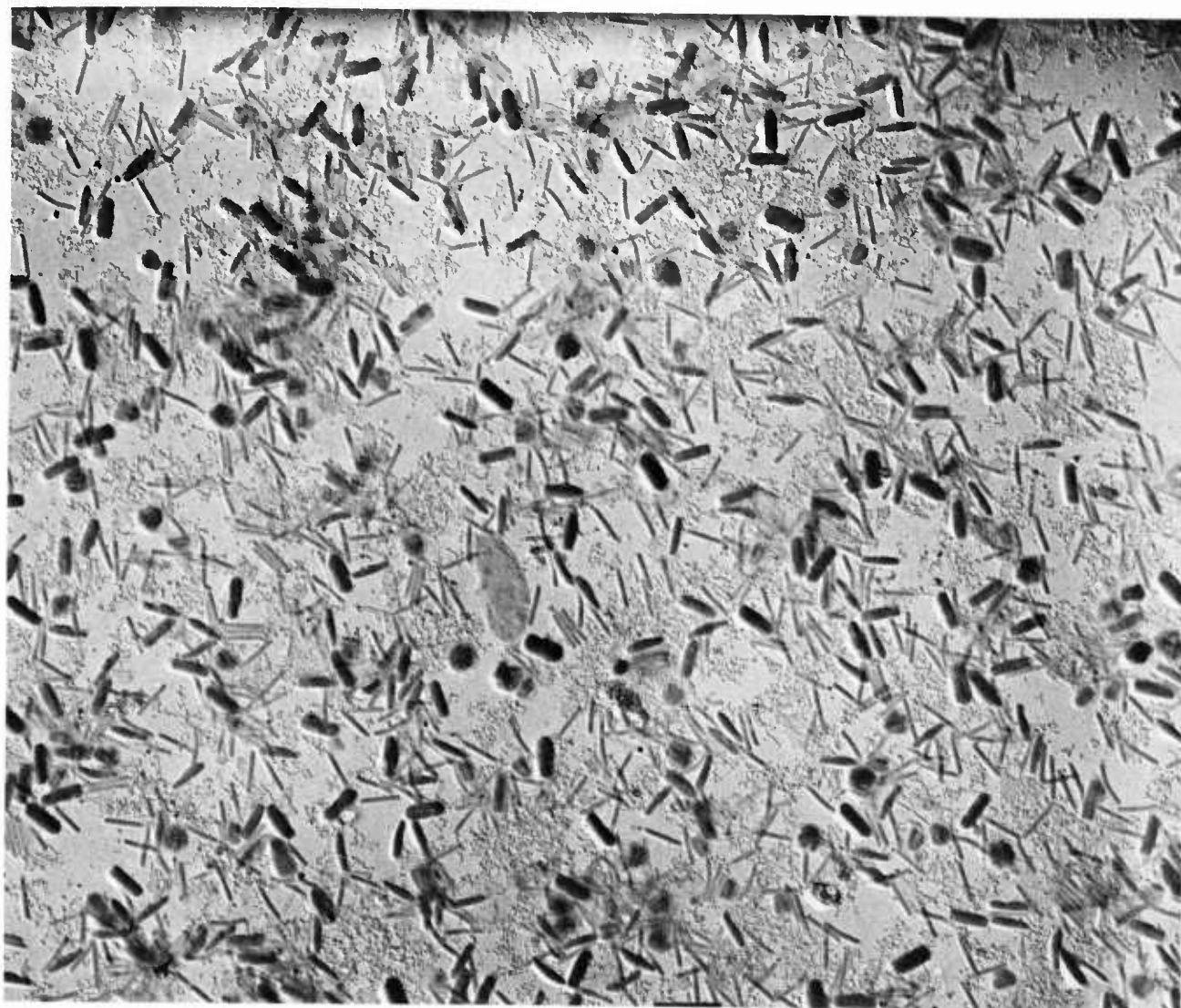
Figure 6.3-20.—*The effect of complete lysis of PIB's. Note different sized rods, which represent the virus particles. (Bar=0.5 μm.)*

Proteins of Polyhedral Inclusion Bodies

After alkaline lysis of the PIB's the proteins are easily separated by differential centrifugation from the free virus rods. The proteins remain in the supernatant while the rods are sedimented to the bottom.

A fraction of PIB proteins from gypsy moth NPV has a sedimentation coefficient of about 12 svedberg units, corresponding to a maximum molecular weight size for the proteins of approximately 275,000 (Bergold 1947).

An amino acid analysis of a fraction of PIB proteins contains the following amino acids with the corresponding mole percent concentration: lysine, 4.6; histidine, 2.8; arginine, 3.6; aspartic acid, 8.8; threonine, 4.5; serine 3.8; glutamic acid, 8.4; proline, 4.8; glycine, 12.3; alanine, 12.4; 1/2 cystine, 0.6; valine,

11.7; methionine, 4.4; isolecucine, 6.9; leucine, 7.1; tyrosine, 5.7; and phenylalanine, 4.7 (Zerillo 1977).

Polyacylamide gel electrophoresis (PAGE) of the PIB protein fraction reveals a major band 5 mm from the origin. The pH of the PIB protein fraction obtained after lysis is about 10.5. If the pH is lowered with dilute acid, material precipitates out of solution (isoelectric precipitation) at certain pH levels. These isoelectric points of precipitation were observed at pH 6.8 and pH 4.5 (Tignor et al. 1976). Isoelectric focusing of the PIB protein fraction, by which the proteins are electrophoresed to their isoelectric point or to where they no longer move in an electrical field, show a major band in the pH range of 6 to 6.5, with 5 minor bands in the pH area 4.7 to 5.8 (Dubois and Kaczmarek 1978).

The subunit size for the PIB proteins was estimated by McCarthy and Liu (1976) by sodium dodecyl sulfate (SDS) PAGE. A major component with a molecular weight of 30,350 comprised 88 to 95 percent of the sample and a minor component with a molecular weight of 62,750 comprised 5 to 12 percent of the sample. These proteins do not give a positive Periodic Acid-Shiff test, which indicates that they contain little if any carbohydrate.

Recently, the PIB proteins of a number of insect viruses have been found to exhibit enzymatic activity, specifically, an alkaline protease. The enzyme functions in an alkaline environment, such as is present during the alkaline dissolution of the inclusion body, and is believed to play a role in degrading the structure of the PIB. A proteolytic activity has been characterized in alkaline carbonate-chloride solubilized proteins from host derived PIB's of the gypsy moth (McCarthy and Liu 1976). This activity has not been detected in carbonate-chloride solubilized protein from PIB's obtained from virus-infected cell cultures of the gypsy moth.

The evidence for an alkaline protease is centered on the number of protein bands observed by SDS-PAGE after a few minutes of dissolution of the PIB and after a longer period of time, 60 to 90 minutes. The number of bands increases with time of dissolution. By applying a proteolytic inhibitor to the dissolution mixture, McCarthy and Liu were able to minimize the degradation of the PIB of gypsy moth NPV, although the pH did not change. If PIB's are boiled, the activity of the enzyme is destroyed, and degradation of PIB's in an alkaline environment proceeds only after considerable time. However, if untreated PIB's are added to the preparation of boiled PIB's, degradation of the treated and untreated particles results (Dubois and Kaczmarek 1978).

The origin of the alkaline protease has not been established; the presence of this activity is not believed due to microbial contamination of host PIB's (McCarthy and Liu 1976).

The enzyme may be identical to the enzyme residing in the gut of the insect that lyses ingested PIB's, liberating the virus rods. However, whether this activity is due to an absorbed host enzyme(s), a nutritional deficiency in the in vitro system, or a basic genetic difference between host-derived virus and tissue culture-derived virus remains to be proven. Another point for further investigation is whether all NPV's possess an alkaline protease in their structure.

For storage and subsequent analyses, PIB proteins should be taken out of their alkaline environment where they are subject to degradation, as noted above, by pH and enzymatic activity. PIB proteins can be precipitated out of solution by pH adjustment towards neutrality, by pelleting after centrifugation at 50,000 for 3 hours, or by salting out of solution with ammonium sulfate to 10 percent of saturation. Ultraviolet absorption analysis of such preparations reveal typical absorption curves for proteins (Mazzone et al. 1971).

Virions of NPV

Virions are contained within the PIB's in the form of rodlike particles. The rods are of similar lengths but vary in width. Free rods may be found outside the PIB's in the hemolymph of the insect larvae, probably the result of a stoppage of the process of including the rods within a PIB brought on by death of individual cells or from the death of the insect. The number of free rods in the hemolymph is small in comparison to the number of rods conatined within PIB'S.

The various rods exist as single, or unenveloped, and enveloped rods. The latter type contain multiples

single rods, as shown by thin sectioning (fig 6.3–21). High-voltage electron microscopy also shows rods of various width existing naturally within a PIB (fig. 6.3–22).

The single rods contain deoxyribonucleic acid (DNA) surrounded by a protein cylinder, which in turn is believed to be covered by the intimate membrane (Bergold 1953). The dimensions of the single rods are 370 nm long and 75 nm wide (Mazzone et al. 1973). The mass of the single rod has been determined by the electron microscopic method previously described. The average mass value of single rods was found to be 1.5×10^{-15} g (Bahr et al. 1976). A convenient way of expressing the mass of viruses is in terms of their molecular weight. The molecular weight is the product of the mass of the virus and Avogadro's number, and for the single rod, its molecular weight was found to be 900×10^{6}, placing it in the class designated as large viruses.

A fraction consisting of the various rod sizes can be obtained after alkaline lysis of the PIB's. The separation of the rod types has been difficult and generally inconclusive as the fractions separated contain all types of rods. It may be that in such preparations, the centrifugation of the rods through a sucrose gradient, as is commonly used, shears away the envelope of some rods, thus producing a variety of rod types at various banding regions along the centrifuge tube. Other procedures for separating the rod types are being tried.

A fraction consisting of the various types of rods can be analyzed by other parameters. When analyzed with the analytical centrifuge, such a fraction contains three sedimenting peaks with sedimentation coefficients of 1,671, 2,580, and 3,322 (Mazzone et al. 1973). The diffusion coefficients were also determined for the rod fraction using laser-light scattering spectroscopy, which measures the diffusion coefficient as a function of the degree of Brownian movement of the particles. The value of the diffusion coefficient of the rod fraction was 2×10^{-8} cm^2 per sec. The ratio of the sedimentation coefficient to the diffusion coefficient is an indication of the molecular weight of the particle analyzed. For the single rod, assuming a sedimentation coefficient of 1,671 and an average diffusion coefficient of 2×10^{-8} cm^2 per sec, the molecular weight is in the hundreds of millions, agreeing with the value obtained by electron microscopy.
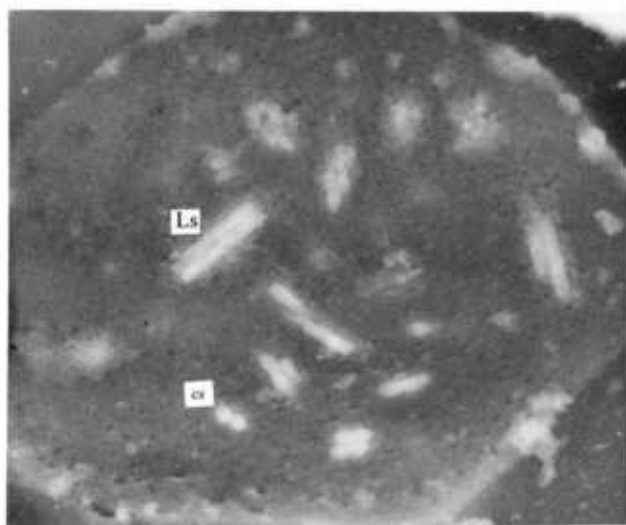
Figure 6.3–21.-*Thin section through PIB showing virions in cross section (cs) and in longitudinal section (ls). (Bar=0.1 μm.)*

Figure 6.3–22.—*High-voltage electron micrograph of an untreated PIB demonstrating that virions of different sizes exist naturally within the PIB. (Bar= 0.1 μm.)*

The rod fraction exhibits a number of morphological anomalies that require explanation. Circular forms were often seen in the rod fraction, and early investigators believed that such forms represented a second type of particle obtained from the lysis of PIB's. Such circular forms probably result from the bending of the ends of the rods into a circular shape, as has been demonstrated for other insect virus systems (Mazzone et al. 1971). Another anomaly seen is a protruding knob at one end, which Bergold (1953) believes may be the point of attachment of the virus particle to the cell, as in the case of a bacteriophage attaching to and infecting a host bacterium. The actual infective process of how rods penetrate cells is unclear, in spite of the large number of studies undertaken on the insect viruses.

The distribution of the rods within a PIB has been stated as being one of random orientation (Bergold 1953). Yet, high-voltage electron microscopy suggests that folds exist in the internal structure of the PIB, in which case the possibility is not ruled out that rods may be laid down in a discrete or ordered arrangement within. Further evaluation would require X-ray analysis of the PIB's.

The enveloped rods, containing multiples of single rods, can be disrupted by the action of chemicals to reveal the single rods contained. In addition to the effect of alkaline pH, which with time degrades the envelope, a high concentration of calcium ions strip away the envelope membrane to yield single rods of essentially the same width and length (fig. 6.3–23).

McCarthy and Liu (1976) lysed PIB's by Bergold's procedure for 3–4 minutes and then lowered the pH to 8. Gradient fractionation of a virus preparation resulted in a multiple-banded sedimentation profile (fig. 6.3–24). Examination of the virus preparation by electron microscopy prior to fractionation showed predominantly (95 percent) enveloped particles (fig. 6.3–25, *A*). The first four fractions were collected and used for protein studies. The remaining bands were not collected, in an attempt to eliminate polyhedral fragments. Particles from each of the first three peaks, after treatment with a detergent (NP–40), consisted of one, two, and three enveloped nucleocapsids, respectively (fig. 6.3–25, *B, C,* and *D*).
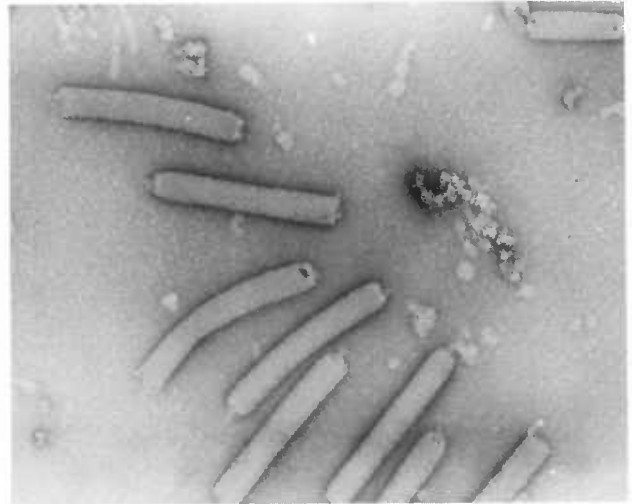
Figure 6.3–23.— *Effect of calcium ions on virus particles. A deenveloping of the particles has resulted. (Bar=0.1 μm.)*
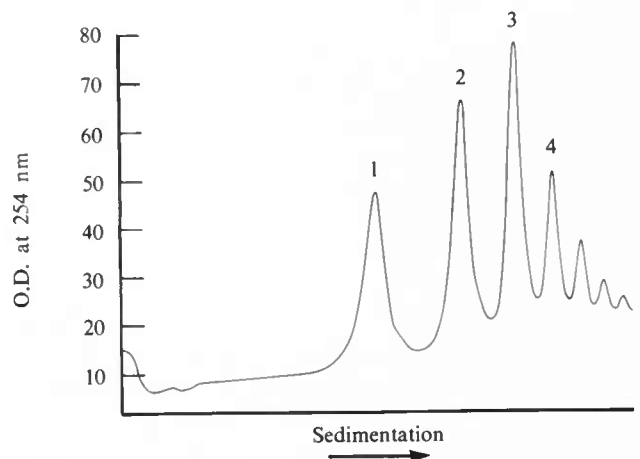
Figure 6.3–24.— *Rate zonal sedimentation profile of the viron fraction in 25–50 percent (w/w) sucrose gradients. Viral peaks 1–4 were collected and used for protein and DNA studies.*

Viral Proteins

Viral proteins were analyzed by both continuous and discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) (McCarthy 1978). Several concentrations of acrylamide were used to estimate the molecular weights of proteins exhibiting very fast and very slow mobilities.
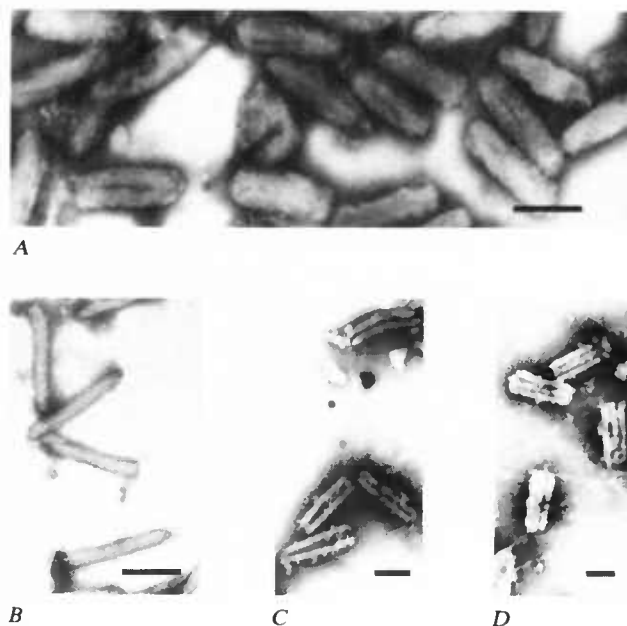
Figure 6.3-25.—*Virus particles purified by sucrose gradient centrifugation:* A, *Particles from gradient peaks 1-4 (see fig. 6.3-24) pelleted by centrifugation;* B, *particles from peak 1 after treatment with a detergent, NP-40;* C, *particles from peak 2 after treatment with NP-40;* D, *particles from peak 3 after treatment with NP-40. (Bar=0.2 μm.)*

Electrophoretic analysis of enveloped virus with the continuous system resolved 11 stained bands with molecular weights from 18,700 to 130,500 daltons. The discontinuous systems resolved approximately 22–24 stained bands with 11 of those stained bands corresponding in molecular weight to the 11 bands resolved in the continuous system. The molecular weight range of the approximately 22 bands resolved in the discontinuous system was from 19,200 to 125,000 daltons. Attempts to detect glycoproteins by the Periodic Acid-Shiff method using gels loaded with 150–200 μg viral proteins were negative. The limit of detection with this method was 1 μg of human glycoprotein (containing 16 percent carbohydrate).

Enveloped virus was treated with a nonionic detergent (NP-40) and fractionated into a soluble fraction and a particulate fraction consisting of deenveloped rods as determined by electron microscopy. Electrophoresis of the soluble fraction in both electrophoretic systems resolved five major stained bands, with a molecular weight range of 41,600 to 92,700 daltons, and several minor bands. The proteins of the nucleocapsid have been difficult to resolve as yet, but the small molecular weight protein (about 18,000 daltons) comprising one of the major proteins of the virus appears to be associated with the nucleocapsid.

Attempts to label the external envelope proteins with NaB_3H_4 resulted in both envelope and nucleocapsid proteins becoming labeled. Moreover, these experiments were run with virus labeled both before and after purification on sucrose gradients; even with the most mild conditions of dissolution and handling, nucleocapsid proteins became labeled. After electrophoresis, approximately 17 labeled peaks were obtained, each corresponding in most cases to one stained viral protein band. In several instances, stained bands close together resulted in one labeled peak. The most heavily labeled bands corresponded to two nucleocapsid proteins with molecular weights of 32,500 and 30,000 daltons. Other heavily labeled bands consisted of three nucleocapsid proteins with molecular weight of 26,000, 23,000, and 13,000 daltons and three NP-40 soluble proteins with molecular weights of 68,000, 63,000, and 60,000 daltons.

Viral Deoxyribonucleic Acid

DNA was isolated by subjecting the viral rod fraction to the action of detergents in the presence of heat. The viral protein breaks down in the process, liberating the DNA which can be further purified by centrifugation procedures. The DNA fraction exhibits a typical ultraviolet absorption spectrum with a maximum at 260 nm and a minimum at 230 nm. The 260 nm to 280 nm ratio was close to 2.0. Mazzone et al. (1973) observed their DNA preparation to be mostly circular with a sedimentation coefficient of 43 svedberg units, corresponding to a molecular weight

of approximately 50 million. McCarthy (1978) found a mixture of three doublestranded DNA species considered to be linear, open circular, and covalently closed molecules. The sedimentation coefficients were 56 and 63 svedberg units for the linear and circular species, respectively. The molecular weight of the DNA ranged from 86 to 104 daltons.

In one DNA preparation, the temperature at which the DNA unstranded or melted, the T_m, was observed to be 92.9, corresponding to a percent guanine plus cytosine (% G+C) value of 57.5 (Camosci et al. 1978). In another DNA preparation, McCarthy (1978) observed a percent G+C of 62.1. These values of percent G+C are in agreement with the value of 57 percent, determined chemically by Wyatt (1952) using base analyses.

DNA isolated from the gypsy moth NPV was found to be infectious when injected into the body cavity of third-instar larvae. When a DNA preparation of optical density 0.3 was injected, 39 percent of the larvae died in 18 days. At the same optical density value, the rod fraction from which the DNA was isolated killed 100 percent of the larvae in 18 days. When the DNA was injected under the same conditions, but at full strength (optical density of 2.8), 72 percent of the larvae died in the same period. The death of the larvae was the result of NPV, as evidenced by the formation of PIB's in the hemolymph and in the blood cells (Mazzone et al. 1973).

Changes in Hemolymph Associated With Virus Infection

A determination of the properties of the hemolymph of the gypsy moth was undertaken (Brown et al. 1977, Brown and Mazzone 1977) in order to acquire knowledge on the healthy and disease states of the insect, especially in terms of viral infection. Such knowledge would allow better understanding of the biochemical and physiological requirements of the gypsy moth and perhaps permit the use of countermeasures to alter such requirements, thereby aiding in the viral control of populations of the insect.

Hemolymph of Healthy Larvae

Larval hemolymph of the gypsy moth is bluegreen and has an absorption maximum at 663 nm and a minimum at 520 nm. The pH of the blood is 6.57, and two sedimenting peaks are observed: A major peak of 17.6 svedberg units, which is 76 percent of the sedimenting material, and a slower sedimenting peak of 7.7 svedberg units representing 24 percent of the sedimenting material. Through use of polyacrylamide gel electrophoresis, 13 bands are present when the gels are stained for protein. Lipids are detected in areas along the gel corresponding to protein bands 13 and 12, and carbohydrates in areas along the gel in all bands, except 6 and 1. As in the case of most insects, trehalose is the principal carbohydrate in gypsy moth hemolymph. Polyphenol oxidase is a major isoenzyme present in the hemolymph. The common amino acids include lysine, histidine, arginine, aspartic acid, threonine, serine, and glutamic acid.

Hemolymph of Virus-Infected Larvae

Larvae infected with NPV undergo a general proteinemia of their normal protein constituents, which can be demonstrated by polyacrylamide gel electrophoresis. From a normal protein banding pattern of 13 bands, a decrease occurs in the intensity of the bands and in the number, as the course of viral infection increases. Lipids and carbohydrates also decrease during the course of infection. One of the last properties to change, ever so slightly, is the ability of the hemolymph to become melanized. Gels showing decreased band numbers consistently demonstrate the presence of polyphenol oxidase. Hemolymph may, with increasing viral infection, show an increase in total proteins, colorimetrically or refractometrically. However, such increase in protein is due to the presence of tissue fragments and cell debris in the hemolymph.

The blue-green color of hemolymph changes with viral infection to white or grayish white as the infection proceeds. The pH of the hemolymph does not change appreciably with infection.

Serology: Immunological Studies of the Baculoviruses

Richard A. DiCapua, James E. Peters, and Philip W. Norton

Introduction

The current emphasis on microbial pesticides is due to the increased environmental concern about chemical pesticides. The immunological and serological characterization of NPV and granulosis viruses (GV) (Baculoviruses) is therefore an area of continuing investigation, with the primary view that labeling and widespread use require exact identification. Considerable progress has been achieved by numerous investigators: Specific serological assays have been developed for several NPV's (Norton and DiCapua 1975) and the presence of Baculovirus group antigen has been confirmed (DiCapua et al. 1978, Norton and DiCapua 1978a, Peters and DiCapua 1978). However, the development of a comprehensive picture of the Baculovirus antigenic complex, or group, subgroup, and type specific antigenicity, has not yet been attained.

Current knowledge of the characteristics of the Baculoviruses, including the mode of virus entry, multiplication, epizootiology, ecology, biochemical, biophysical, and morphological properties and nomenclature led David (1975) to state "... this genus is the best known of insect viruses." However, increasing knowledge, if devoid of true serological characterization, will not satisfy the mandate for safe and effective implementation of biological pesticides, and efforts will remain incomplete until the number of assumed distinct viruses is verified. Establishing defined serological interrelationships is, therefore, essential and must include a determination of the serological relationships between insect and noninsect viruses that may multiply in both insect and noninsect hosts, particularly man. Recalling the advent of commercial vaccines (polio, measles, and influenza, for example) and the difficulties that occurred at various developmental stages emphasizes the proven value of serological characterization.

Basic serological investigations of the Baculoviruses have been conducted by Aoki and Chigasaki (1921), Gratia and Paillot (1938,1939), Krywienczyk and Bergold (1960a, b, c, d, 1961), Tanada (1954), and others. More recent descriptions of the Baculoviruses have been provided by Smith (1967), Bellet et al. (1973), and David (1975).

Immunochemical Considerations

Serological characterization of any antigen and the consequent antibody response is dependent primarily upon the molecular conformation, display, and accessibility of antigenic determinants. Proteins possess a finite number of these determinants, and conformational changes, occurring through denaturation or chemical alteration, result in changes in antigenicity. NPV's possess complex antigenic structures that can be modified or altered by solubilization and extraction techniques that are routinely employed in laboratory studies. Alternatively, PIB's undergo degradation in vivo, via immunization, thereby exposing internal antigenic components that may not be present in in vitro degraded products. Consequently, responses to a given antigen may vary among the species chosen for immunization, anticipated positive and negative reactions may prove to be reciprocal, and antigens derived in the laboratory may prove to be different in specificity to those found in their natural habitat. These variations must be accounted for if an accurate serological characterization of the Baculovirus or any other virus group is to be attained.

Polyhedrin and granulin (matrix protein, PIB protein) are major structural proteins of polyhedra and granules, which form a crystalline lattice surrounding and occluding virions. These polymerized NPV or GV proteins have antigenic determinants at the surface of both monomers and polymers referred to as metatopes. These surface determinants may become masked by the interaction of monomers to form polymers, in which case they are referred to as cryptotopes, or contribute to the formation of new determinants (neotopes) through

monomer juxtaposition (for a review of protein conformation and antigenic structure, see Crumpton 1974 and Neurath and Rubin 1971). Therefore, varying degrees of polyhedrin or granulin aggregation can provide a significant impact upon the antigenicity of proteins or polypeptides isolated for serological study. Inhibition of the protease-like activity associated with in vivo viral protein fractions (Eppstein and Thoma 1975, Kozlov et al. 1975, McCarthy and Lui 1976, Summers and Smith 1976) may therefore preserve metatopes. Allowing proteolysis to occur may be necessary for crytotope exposure, and finally, some antigens may prove to be resistant to proteolysis and are therefore present in both degraded and non-degraded fractions (Norton and DiCapua 1978a, b).

Virions, like the PIB's and granules, possess antigen determinants found within membrane structures, nucleic acids, and associated proteins. Differing isolation conditions, such as variations in salt concentrations, may modify the antigenic complex of the virion, by altering the confirmation of envelope proteins or by their selective removal. Capsid or core proteins may also be aggregated or polymerized and therefore be subject to the same considerations cited for the antigenic determinants of polyhedrin or granulin. Mercaptoethanol and sodium dodecylsufate (SDS) treatment of proteins in preparation for polyacrylamide gel electrophoresis (PAGE) may also affect antigenic determinants. Obviously, with the true number of antigenic determinants unknown, the current methods of antigenic preparations for serological characterization of the Baculoviruses must undergo continuous reevaluation if erroneous judgments are to be avoided.

Baculovirus Antigen Preparation

Serological characterization of the NPV's and GV's requires the solubilization of polyhedra and granules. The procedure most frequently cited is that of Bergold (1947, 1953, 1958, 1963) and consists of the dissolution of inclusion bodies at an alkaline concentration (0.005–0.02 M Na_2Co_3 in 0.05 M NaCl) appropriate for the harvesting of polymerized protein

solutions with minimum denaturation. The dissolution is followed by centrifugation and subsequent separation of virions and membrane fractions from the polyhedrin or granulin.

This procedure is fundamental to the serological, immunoelectrophoretic, and immunofluorescent studies of Krywienczyk (1962, 1963, 1967), Krywienczyk et al. (1958), and Krywienczyk and Bergold (1960a, b, c, d) and provides a standard comparison for their work. However, the weak alkaline pH described by Bergold (1953) and redefined by Krywienczyk (1960b) to be a suitable pH *below* 9 is not utilized by all investigators who cite the method. Modifications of the Bergold method are, of course, appropriate and, when cited properly, add to general knowledge revealed by the experiments of Harrap and Longworth (1974), Hukuhara and Hashimoto (1966), Longworth et al. (1972), Norton and DiCapua (1975), Peters and DiCapua (1978), Scott and Young (1973), and Shapiro and Ignoffo (1970).

The presence of an alkaline enzyme, of endogenous or exogenous origin, in polyhedrin or granulin (Eppstein and Thoma 1975, Kozlov 1975) with high buffering capacity will generate dissolution product variations via the Bergold procedure. These variations will alter the native state of the occlusion and virion proteins, result in unanticipated changes in the antigenicity of the byproducts (see section on Immunochemical Considerations), and consequently invalidate the comparison of serological data from various laboratories. It is necessary, therefore, to determine to what extent prior serological investigation for Baculoviruses utilized pure or, at minimum, the same antigens. For example, the serological comparisons cited earlier have generally utilized antigenic preparations separated by differential centrifugation, and therefore the occlusion and virion fractions obtained probably contain mixtures of both. Under such conditions, one cannot distinguish between cross reactions due to true common antigenicity of these fractions or contamination. Harrap and Longworth (1974) first identified this problem, providing procedures for valid biophysical, biochemical, and immunological comparisons of these viruses.

These variations in dissolution conditions are further compounded by other variations. The interval between solubilization and inoculation, the manner of storage, the presence of enzyme inhibitors, bacteriocidal or bacteriostatic agents, haptens, host proteins, etc., are all factors known to affect the antigenicity of any given substance.

Comparisons of short- and long-term dissolution periods for the solubilization of PIB's of various NPV's have revealed that increased degradation of polyhedrin occurs with increased dissolution time. This is easily verified by SDS–PAGE, where all samples assayed are kept constant with respect to pH, ionic strength, and polyhedrin concentration. Comparing the number of precipitates formed in immunodiffusion assays between degraded and nondegraded polyhedrins, for the purpose of identifying changes in antigenicity, indicated that:

• The Baculovirus polyhedrin group specific antigen (BP-gs), identified by Norton and DiCapua (1978), is present in both degraded and nondegraded protein—that is, the group antigen is resistant to carbonate treatment and exogenous protease activity.

• Polyhedrin derived from NPV's isolated from ovarian cell culture systems contains BP-gs but lacks protease activity.

• With *Lymantria dispar*, there are several Baculovirus-polyhedrin type-specific (BP-ts) antigens that are apparently cryptic (nonfunctional in nondegraded polyhedrin), that require protease activity for exposure, and that are susceptible to total protease degradation (Peters and DiCapua 1978).

Obviously, rigorous characterization for the purpose of defining that antigenic structure of the Baculoviruses requires standardized procedures for antigen isolation. Kawanishi and Paschke (1971) compared the relationship of buffer, pH, and ionic strength on the yield and infectivity of virions obtained upon dissolution of *Rachoplusia ou* NPV. Their protocol could be easily applied for measuring the effect on antigenicity of native virus degraded polyhedrins or virion proteins, thus allowing for proper manipulation of the variables.

Preparation of Baculovirus Antibody

In many investigations, the production of specific antibody in laboratory animals and its isolation are problems of practical and academic importance. The dissolution procedure for obtaining polyhedrin, granulin, and virions to be used as immunogens is not standardized. "Immunogen" in this context is simply a potential immune-response stimulator, since its identity or purity is usually unknown at the time of inoculation. Variations in the Bergold procedure are often matched by methods reported for the production of antibody, and when unreferenced, these procedures are meaningless. Injection schedules and routes of inoculation that allow for duplication must be devised (Norton and DiCapua 1975) and continually reevaluated if the investigator is to make valid decisions.

Since each investigation is usually unique, strict immunological guidelines cannot be provided, and general guidelines appropriate for all types of antibody desired would be exhaustive. However, the methods employed for antiserum production and the choice of assay are matters of great importance. Procedures and rationales have been provided (Campbell et al. 1964, Harper and Martos 1973, Hyde et al. 1967, Moreland 1965, Ouchterlony 1962, Pike 1967) and serve as examples to be followed to obtain an accurate serological characterization of the Baculoviruses.

Serological Characterstics of Polyhedrin and Granulin

Serological relationships among PIB's of different hosts were observed first by Gratia and Paillot (1939), who proved that no relationship existed between *Bombyx mori* and *Euxoa segetum* Schiffermuller polyhedrins. Bergold and Friedrick-Freska (1947) demonstrated that the polyhedrins of *L. monacha* and *L. dispar*, although unrelated to *B. mori*, are interrelated.

Norton and DiCapua (1975) have demonstrated that the polyhedrins, and possibly the virions, of *L. dispar* and *Neodiprion sertifer* are serologically related by employing multiple injections of immunogen in excess of 3 mg protein in rabbits.

The use of either rabbit or guinea pig antisera to *L. dispar* polyhedrin has produced type I reactions (identity) with the polyhedrins of 15 NPV's (table 6.3-17), demonstrating the presence of a group antigen common to these Baculoviruses (Norton and DiCapua 1978*a*), designated BP-gs (see Baculovirus Antigen Preparations). *Spodoptera frugiperda* polyhedrin, at equivalent concentration, does not react with *L. dispar*, suggesting that its group antigen is, at such concentration, unsuitable for precipitation or is sterically hindered. Regardless of which of these two explanations is most valid, its group antigen is known to be present via its specific neutralization capacity (Norton and DiCapua 1978*b*).

Serological cross-reactivity has been observed between homologous and heterologous polyhedrins and virions with antisera to either Lepidopterous or Hymenopterous NPV's. Similar observations have been reported by Longworth et al. (1972), who detected the presence of antigens A and B in the granulin of *Pieris brassicae*. Antigen B was found on the surface of the enveloped virus particle and in the PIB "envelope." Antiserum to purified virions detected the B antigen only in granulin.

Scott and Young (1973), on the basis of their assessment of *Trichoplusia ni* NPV, observed five

Table 6.3-17.—*NPV and GV with (Baculovirus/Polyhedrin group specific) activity demonstrated by antipolyhedrin or antigranulin*

Polyhedrins	Granulins
Arctia caja	*Malacasoma disstria*
Autographa californica	*Pieris brassicae*
Bombyx mori	
Heliothis zea	
Lymantria dispar	
L. monacha	
Neodiprion sertifer	
N. taedae	
Orgyia leucostigma	
O. pseudosugata	
Platynota idaeusalis	
Pseudoplusia includens	
Spodoptera frugiperda	
Tipula paludosa	
Trichoplusia ni	

precipitates between the virion fraction and its homologous antiserum. Since one of the antigens is also found in the polyhedrin, they suggest its presence could be due to degradation during polyhedra dissolution, which may also account for the cross-reactivity reported by Longworth et al. (1972). If the observation of cross-reactivity between polyhedrin and virion can be resolved as contamination or true shared antigenicity, then the nature of the polyhedrins and granulins of Baculoviruses, intrinsic to the viral genome or possible host-defense byproducts, can be further reflected upon. The necessity of an appropriate dissolution procedure is therefore reemphasized.

The first investigation of the serological relationship between the granulosis viruses and NPV's was conducted by Tanada (1954), who found the two PIB types of *P. rapae* to be related. Krywienczyk and Bergold (1960*b*), on the basis of their studies of *Recurvaria milleri* granulin, reported a strong serological relationship with *B. mori, Malacosoma disstria, Choristoneura fumiferana* (Clem.), *Colias philodocererytheme, M. americanum*, and *L. dispar*. Such cross-reactions could be due to the presence of a common C antigen found by Croizier and Maynadier (1973*b*) in polyhedrin and granulin via carbonate or thioglycolate dissolution. Their study also confirmed the presence of a T antigen in the granulin of *P. brassicae* and a B antigen in the polyhedrin of *B. mori* via thioglycolate dissolution only, stressing again the effect of dissolution conditions on antigen variation.

Tanada and Watanabe (1971) observed the presence of common antigens in the granulins of two strains of *Plodia unipuncta* that are distinguished by synergy and pathogenicity. They also describe the effect that protein incorporation in the capsules could have—for example, the establishment of a false serological relationship based upon shared antigenicity of host contaminants.

Glaser and Stanley (1943) detected cross-reactivity between antipolyhedra and the "healthy" hemolymph of *B. mori*. One interpretation, based upon the experiments of Aizawa (1954) and Krywienczyk and Bergold (1961), is that the reaction was due to

antihemolymph activity. However, Young and Johnson (1972) detected virus specific soluble antigens in the fat bodies of infected *T. ni* larvae. The presence of such antigens in the hemolymph (perhaps via a fat body hemocoel access) provides an alternative explanation of Glaser and Stanley's results, implying that the hemolymph was, in fact, infected. This serves as a reminder of the absolute need for infectious agent-free controls.

The cross-reactivity of homologous antisera to *N. sertifer* virions, the reciprocal cross-reactivity of antisera to viral components of *L. dispar* and *N. sertifer*, and, via hemagglutination-inhibition, the cross-reactivity of antisera to polyhedra, polyhedrin, and virions of *N. sertifer* with the hemagglutinin of *L. dispar* polyhedrin (Norton and DiCapua 1975) have all been demonstrated. Although the cross-reactivity between polyhedrins and virions of homologous origin may be due to contamination, the cross-reactivity between antisera to the polyhedral components of *N. sertifer* and the polyhedrin of *L. dispar* clearly demonstrates that such reactions are not exclusive to Lepidopterous viruses as previously reported (Krywienczyk and Bergold 1960*d*) and that a serological relationship exists between two viruses that have highly divergent hosts.

Hemagglutinin activity has also been found in the polyhedrin of *Spodoptera frugiperda* NPV by Reichelderfer (1974), in the virion fractions of the CPV and NPV of *B. mori* by Miyajima and Kawasi (1969), and in the NPV of *Heliothis zea* by Shapiro and Ignoffo (1970). Because it is difficult to distinguish the polyhedrins of *L. dispar* and *S. frugiperda* by immunodiffusion assay, the polyhedrin hemagglutinin activity of these two NPV's has significant diagnostic value (Norton and DiCapua 1975).

Serological Characterization of Virions

Serological analysis of Baculovirus virions is inherently more complex because of the higher number of potentially distinct protein moieties. Young and Lovell (1973) observed 12 polypeptides in *T. ni*

virions, and Padhi et al. (1975) observed 14 polypeptides in *L. dispar* virions via SDS–PAGE. However, antisera to these virions do not form 12 and 14 precipitates respectively. Scott and Young (1973) were able to demonstrate five precipitates with *T. ni* virions, and in this program at least five precipitates were observed with the *L. dispar* virions. The number of protein bands present in the acrylamide gels cannot be directly correlated with the number of antigenically distinct virion proteins. It is therefore possible that these antisera contain specific antibodies to more than five virion antigens that will not precipitate with their homologous antigens in a state of antigen excess. Consequently, more sensitive assays, such as a radioimmune assay (RIA), are required.

Analysis has been attempted of reciprocal cross-reactions between antisera and virions of *L. dispar* and *N. sertifer* NPV's by immunodiffusion. Heterologous precipitation was present but difficult to detect; therefore, heterologous antigens were assayed for cross-reactivity by the intragel absorption technique (Feinberg 1957). Specific wells were preloaded with antisera, and after complete diffusion, antigen was loaded into the same wells. With this system, the number of precipitates between *L. dispar* virion and homologous antisera was decreased if the antigen well was first filled with antisera to *N. sertifer* virion. The absence of precipitates between homologous antigen and antibody by absorption with heterologous antibody verifies the presence of a common antigen(s) between these NPV's.

The fundamental basis for cross-reactivity between virions and their polyhedrins or granulins has not been resolved. Polyhedrin antisera, stimulated by immunogens from polyhedra rigorously cleaned and dissolved under restrictive conditions of pH, temperature, and time, cross react with virions isolated from sucrose gradients after ultracentriguation. Because contamination was minimized, it appears that there is evidence to support the concept of a common antigen between virions and polyhedrins. Such an antigen may function in vivo as a receptor for polyhedrin monomers and as an

initiator of polyhedrin polymerization for PIB formation.

Group Antigen in Polyhedrin

Serological cross-reactions between polyhedrins and granulins from a large number of NPV's and GV's were investigated by Krywienczyk et al. (1958, 1960a, b, c, d, 1961) and Tanada (1954). Krywienczyk et al. concluded that serological groups exist among insect viruses, namely the NPV's of Lepidoptera, the NPV's of Hymenoptera, and the GV's of Lepidoptera.

Studies of polyhedrin fractions from numerous NPV's (table 6.3-17), which used several different sources of antisera to polyhedrin, indicated that the serological cross-reactivity between the polyhedrins from NPV's of Lepidopterans and Hymenopterans is due to the presence of BP-gs, a group antigen common to all Baculoviruses assayed to date (Norton and DiCapua 1978a) and determined to be resistant to PIB protease activity (Norton and DiCapua 1978b).

Gypsy Moth NPV Studies: Current Status

Host-derived *L. dispar* NPV PIB's, purified by K-rotor centrifugation and treatment with urea and sodium dodecyl sulfate to remove nonviral contaminants, have been the principal source of all antigens utilized. Polyhedrin and virions have been released from their inclusion bodies by the method of Bergold (1953) and isolated by high speed and sucrose gradient centrifugation. Antisera to *L. dispar* NPV antigens and *L. dispar* hemolymph were raised in 11- to 13-kg New Zealand white rabbits or 250- to 300-g guinea pigs. These animals were divided, per experiment, into four antigen groups (PIB, polyhedrin, virion, and hemolymph). Usually each group consisted of four animals (minimum of two) with half the animals in each group receiving antigen plus Complete Freund's Adjuvant® (Difco, Baltimore, Md.), the other half antigen only. The initial injection schedules, antigen doses, routes of inoculation, and bleeding schedules are presented in table 6.3-18. Recent investigations requiring the production of antisera have employed the method of Vaitukaitus et

al. (1971), which requires by comparison minimum quantities of immunogen (3-5 mg total protein) to achieve comparable antiserum titers.

These antigen and antiserum reagents have been examined for specificity by a series of standard immunological assays or modifications thereof, identified in table 6.3-19.

L. dispar NPV polyhedrin contains hemagglutinating activity for chicken erythrocytes (Norton and DiCapua 1975) that specifically identifies the NPV of the gypsy moth from those Baculoviruses listed in table 6.3-17. At equivalent concentrations and under identical assay conditions, the polyhedrins of these nongypsy moth NPV's failed to agglutinate the chicken erythrocytes. None of the polyhedrins assayed possessed hemagglutinins for any other species of erythocyte assayed (table 6.3-20). The chemical structure of the *L. dispar* polyhedrin receptor on the chicken erythrocytes has been identified and its protein-carbohydrate interaction discussed (Peters and DiCapua 1978).

Inhibition of the hemagglutinating activity of *L. dispar* polyhedrin for chicken erythrocytes can be accomplished with antisera to the polyhedrins of heterologous NPV's. For example, anti-*Neodiprion sertifer* polyhedrin will neutralize the hemagglutinating activity of *L. dispar* polyhedrin, demonstrating, by reciprocal activity, the presence of at least one antigen in common between *N. sertifer* and *L. dispar* NPV's. (Norton and DiCapua 1978) However, no heterologous antisera can inhibit the hemagglutination to the same degree as anti-*L. dispar* polyhedrin at equivalent concentrations. Consequently, *L. dispar* polyhedrin can be identified from a battery of unknown Baculovirus polyhedrins not only by its hemagglutinin activity but also by its reciprocal hemagglutination-inhibition activity. In the same manner, the neutralizing activity of *L. dispar* polyhedrin or anti-*L. dispar* polyhedrin and virion can be assessed by the immunoflourescence, intragel absorption, immunodiffusion, and mixed hemagglutination assays. The latter assay, involving the combination of infected gypsy moth hemocytes and guinea-pig erythrocytes, is particularly useful (and

Table 6.3-18.—*Injection schedule and routes of injection for the NPV antigens of* Lymantria dispar[1]

Antigen group	Day	Route	Dose	Dose concentration	Number of animals	Trial bled[2]
PIB & CFA	1, 4	IM	4 ml	5×10^5/ml	2	7, 10, 20
PIB	1, 4	IM	2 ml	1×10^6/ml	2	7, 10, 20
PIB	8, 11, 15	IM&SC	2 ml	1×10^6/ml	∅	
PIBP & CFA	1, 4, 7	IM	2 ml	20 mg/ml	2	
PIBP	1, 4, 7	IM	1 ml	40 mg/ml	2	
PIBP	11	IM&SC	2 ml	40 mg/ml	∅	7, 10, 20
PIBP	14	IM&SC	4 ml	40 mg/ml	∅	
RODS & CFA	1, 4, 7	IM	2 ml	20 mg/ml	2	
RODS	1, 4, 7	IM	1 ml	40 mg/ml	2	7, 10, 20
RODS	11	IM&SC	2 ml	40 mg/ml	∅	
RODS	14	IM&SC	4 ml	40 mg/ml	∅	
PdH & CFA	1, 4, 7	IM	2 ml	12.4 mg/ml	2	
PdH	1, 4, 7	IM	1 ml	25 mg/ml	2	7, 10, 20
PdH	11	IM&SC	2 ml	25 mg/ml	∅	
PdH	14	IM&SC	4 ml	25 mg/ml	∅	

[1]CFA = Complete Freund's Adjuvant®; IM = intramuscular; SC = subcutaneous; PdH = *P. dispar* hemolymph; ∅ each animal within group.

[2]Days post last inoculation.

Source: Norton and DiCapua 1975.

sensitive), because the donors of antiserum to *L. dispar* polyhedrin and erythocytes can be the same animal. This is important because a single donor eliminates the possibility of false positive reactions based on antierythrocyte activity.

Cross-immunoelectrophoresis and immune-absorption electrophoresis have extended knowledge of the *L. dispar* virus antigenic structure beyond the primary goal of specific identification of the gypsy moth NPV. Utilizing antisera to *L. dispar* polyhedrins, the cross-immunoelectrophoresis assay verifies the presence of at least five distinct antigens. This is in agreement with the number of precipitates observed in immunodiffusion assays and allows for the identification of the relative electrophoretic mobilities of each antigen and the identification of the fraction containing the BP-gs activity (see section on Baculovirus Antigen Preparation). The characterization of *L. dispar* polyhedrin in this manner is prerequisite to the isolation of one or more type specific antigens of *L. dispar* NPV, the ultimate goal.

The use of heterologous antisera to *L. dispar* polyhedrin in the immune-absorption electrophoresis

Table 6.3-19.—*Immunological assays utilized for the identification of* Lymantria dispar *(NPV)*

Immunodiffusion (Ouchterlony)
Intragel absorption
Complement fixation (CF)
Immunofluorescence (FA)
Immunoperoxidase
Cross-immunoelectrophoresis
Immune-absorption electrophoresis
Mixed hemagglutination
Hemagglutination
Hemagglutination-inhibition

Table 6.3-20.—*Erythrocyte donor species lacking hemagglutinin receptors for Baculovirus polyhedrins assayed*

Human	Pig	Rabbit
Horse	Rat	Guinea pig
Cow	Mouse	Turkey

assay provides a means of eliminating the activity of BP-gs antigenicity, thus the polyhedrin can be assessed for potential subgroup and type-specific antigenicity. For example, because antisera to *Spodoptera frugiperda* polyhedrin contains a specific antibody to BP-gs, it can be used to absorb out BP-gs antigens from *L. dispar* polyhedrin. Consequently, the residual antigens, separated by electrophoresis, can be reacted with both homologous and heterologous antipolyhedrins. Therefore, subgroup and type specific antigens can be identified by process of elimination. *L. dispar* polyhedrin assayed in such a manner appears to have more than one type-specific antigen and may have as many as four. This knowledge justifies current attempts to devise fractionation procedures appropriate for the isolation of any given type specific antigen from *L. dispar* polyhedrin or virion. Once isolated, the monospecific antigen can be utilized as immunogen for the production of monospecific antisera—that is, a reagent that will react exclusively with the viral components of the gypsy moth NPV.

Conclusion

Comparison of various investigations of the serological interrelationships of the Baculoviruses leads to several interpretations. The data of Krywienczyk and Bergold (1960c) provide for the division of these viruses into three groups: The granuloses of Lepidoptera, the nucleopolyhedroses of Lepidoptera, and the nucleopolyhedroses of Hymenoptera. Bellet (1969) has statistically analyzed the above groups, and on the basis of the molar proportions of guanine and cytosine in their DNA, groups the viruses as follows; The granulosis virus of *Choristoneura fumiferana* (Clem.), the nucleopolyhedroses of Hymenoptera, and the nucleopolyhedroses and granuloses of Lepidoptera. Alternatively, when the data of Croizier and Meynadier (1973) are compared with those of Norton and DiCapua (1975, 1978*a, b*), Peters and DiCapua (1978), and DiCapua and Norton (1976, 1977), a serological relationship based on common antigenicity exists between the nucleopolyhedroses and granuloses of Lepidoptera and Hymenoptera

that may allow for discrimination among individual viruses independent of host phylogeny.

Efficacy

Franklin B. Lewis and William G. Yendol

Introduction

Wilt disease was first noticed in gypsy moth larval populations in the early 1900's in both the United States and Europe (Glaser 1915). In 1942 Bergold (1958) identified the causative agent of this wilt disease to be a nucleopolyhedrosis virus. Following these early investigations, many field and laboratory studies have been undertaken concerning the virus-host interactions (Wallis 1957, Campbell 1963, Turner 1963, Rollinson et al. 1965, Magnoler 1968, Doane 1969, Wollam et al. 1978, Yendol et al. 1977).

Bess (1961), after conducting studies on the population ecology of the gypsy moth, concluded that the NPV infecting the larvae was only a minor factor in regulating gypsy moth populations. However, through the use of life tables, Campbell (1961) demonstrated that the virus was a primary influence on the survival rate of late-instar larvae in dense populations, and these dense populations of the gypsy moth often declined because of viral epizootics (Campbell 1963, 1964).

Vasiljevic (1961), investigating the gypsy moth in Europe, reported 98–100 percent mortality in larvae from areas where an epizootic of NPV had attained a maximum level.

In spite of evidence supporting the fact that natural virus epizootics appear to regulate gypsy moth populations at high densities, some investigators (Magnoler 1968*b*, Rollinson et al. 1965) have indicated that applications of NPV to field populations gave only limited effectiveness as a control agent.

What follows is a sequential chronology of laboratory and field tests to evaluate the efficacy of the gypsy moth NPV. The initial field testing was done at very high doses based on the data of Rollinson et al. (1965) and was conducted using a truck-mounted mist

blower. Ground application was first utilized because greater deposit and thus greater effect were expected.

Evaluation Techniques

Generally applicable techniques for the evaluation of microbials used against the gypsy moth have been discussed by Connola et al. (1966) and Lewis (1977). There are several points regarding the measurement of efficacy of NPV against the gypsy moth that should be understood.

• NPV is slow acting, showing its effect 10–15 days after application. This is in contrast to the quick action of classical synthetic pesticides.

• The use of NPV does not affect other natural mortality factors or pests other than the gypsy moth.

• Natural NPV can cause mortality in the treated populations, usually after the primary effect of applied NPV.

Because of these situations, it is necessary to use techniques that allow for the measurement of larval mortality due to the NPV treatment and to separate, where possible, the effects of mortality due to factors other than the applied NPV. Techniques such as timed walks to count larvae, frass traps, caged larvae, larval rearings, and foliage bioassay have been used to measure the direct effects of NPV.

There are two levels of evaluation used in assessing the effects of NPV on gypsy moth. The primary level of evaluation assesses foliage protection and population change. The secondary level of evaluation measures direct effects on the larvae and involves timed larval counts, larval counts under burlap strips, and examination of larvae for NPV kill.

Primary Level of Evaluation

Foliage Protection

Visual ground estimates of defoliation are made on individual oaks and other tree species in each fixed and variable prism point (Wilson and Fontaine 1978). These estimates are made at the conclusion of the larval feeding period and prior to refoliation time. Both total defoliation and net defoliation (final minus

beginning defoliation at time of spray) are used. Complications can arise when significant numbers of defoliators other than gypsy moth are present.

Population Change

Population change is evaluated on the basis of egg-mass change from prespray levels to postspray levels. Egg-mass counts are made utilizing the prism-point sampling technique. Additional information is taken on numbers of eggs per mass and viability of eggs, both prespray and postspray. These tests determine if unusual hatch percentage, due to parasitism or winter kill, has occurred prior to spray or if carryover effects of the NPV can be noted in the next generation.

Secondary Level of Evaluation

Timed Larval Counts

In treated and control plots, 5 or 10 minute counts are made two to three times weekly after treatment. These counts give relative population densities and provide information on the effect of the treatment. In particular, they allow the detection of unexpected population collapse or reinvasion of healthy larvae from outside the test blocks.

Burlap-Band Larval Collections

Counts of living and dead larvae are made under burlap bands placed on selected trees in the experimental blocks. Dead larvae are examined for the presence of NPV by microscopic examination to ensure that mortality is being caused by the NPV treatment. Natural NPV mortality in control blocks is estimated in this manner.

Effect of Gypsy Moth Population Density

Dense gypsy moth population (above 2,500 egg masses per 0.4 ha) can crash due to natural factors (obliterating treatment effects). They also give rise to large larval populations that cause early damage to leaf surfaces, leaving little for NPV deposit. Reinvasion of treated plots by large larvae later in the

feeding period can also occur with dense populations. We have therefore restricted NPV efficacy tests to population densities from 300 to 2,000 egg masses per 0.4 ha. Data accumulated in the various field tests indicate that NPV works best at low to medium densities and thus could be used in the earlier stages of the population buildup rather than at peak densities.

The size of plots used to evaluate the efficacy of gypsy moth NPV is important for one reason: Because of the fast degradation of the NPV under the influence of solar radiation and other environmental effects, larvae ingesting foliage a week or more after application will not be infected If the treated plot is too small (fewer than 14–15 ha), untreated larvae from outside the treatment can and do invade the treated plot and survive. This reinvasion can drastically confound the true effect of treatment. It is necessary to minimize this effect by treating plots large enough to prevent reinvasion from interfering with the evaluation or by making measurements near the center of the treated plots where reinvasion problems would be minimal.

Formulation and Application

With respect to microbials, this entire area has not received the attention that it deserves. The importance of formulation-application is just now being addressed (Ignoffo 1978, Smith et al. 1977, Lewis 1977, Boving et al. 1971).

NPV's, including that of the gypsy moth, have certain unique characteristics that must be taken into account when using them for control of insects: (1) NPV must be eaten to be effective, thus the feeding activity of the target pest must not be impaired; (2) NPV is quickly degraded by ultraviolet, thus the formulations must provide protection from ultraviolet; (3) the feeding area must be thoroughly covered, including both surfaces of the leaves, to maximize ingestion of the virus; (4) leaf expansion should be well advanced to ensure minimal untreated expansion areas; (5) the coverage of the target area must remain in place for several days to ensure contact between the NPV and the host insect; and (6)

the susceptibility of the gypsy moth larva decreases rapidly as it grows larger.

Because NPV must be eaten to be effective, water has been used as the spray diluent. The use of water presents some problems of considerable complexity that involve evaporation, source, pH, and organic and inorganic contaminants. The substitution of water by another diluent with little evaporation problems and no feeding deterrency or phytotoxicity is needed.

Certain stickers and spreaders are very effective but can cause problems if they prevent the polyhedra from breaking down in the insect gut, freeing the virions that cause infection.

Conventional application equipment has been used to apply the gypsy moth NPV. Somewhat better results have been obtained when motorized spinning cylinder nozzles are used in place of flat fan nozzles. Much more attention needs to be applied to the development of proper equipment for the application of microbials.

Spray assessment techniques have generally been confined to spray cards and bioassay of treated foliage. A handbook for spray assessment (Dumbauld and Rafferty 1977) has recently been issued, but no reference is made to the assessment of biological spray material such as the microbials.

Ground Application of Virus

In 1972, two experimental sites located in Schuylkill County, Pa., were sprayed from the ground with NPV at the rate of 1×10^{13} PIB's per 0.4 ha per application (Yendol et al. 1977). Each site received two spray applications, 10 days apart. The initial spray was applied when at least 50 percent leaf expansion had occurred among the white oaks, *Quercus alba* L., and when 50 percent or more of the larvae had hatched.

In one of the treated sites (dense population) a natural virus epizootic occurred that subsequently resulted in a population collapse the following year.

The other virus-treated site (low-medium density) that was artificially infested sustained 15 percent defoliation. Defoliation ranged from 15–20 percent in the controls. These virus-treated plots had a postspray

egg-mass mean of 52 per 0.1 ha., and the corresponding untreated plots a mean density of 823 egg masses per 0.1 ha.

The encouraging results of the use of NPV by ground application were used to plan aerial applications scheduled to be initiated in spring 1974. This test also gave indications of the dangers of dense population collapse and also the first intimation that the NPV effect in low to medium population densities would be of an acceptable level. Accumulation of additional virus and safety data was done in 1973.

Field Persistence of Selected Virus Formulations

An important consideration in establishing the efficacy of the gypsy moth virus has been the lack of field persistence of the formulations used.

In 1972, a field persistence test using several potential formulations was conducted in Centre County, Pa.

Chestnut oak, *Quercus prinus* L., and red oak, *Q. borealis* Michx., 6–9 m in height, were the tree species utilized. The test site was composed of 36 isolated trees or isolated small groups of oaks having a ground surface canopy mean area of 134.7 m^2.

Foliar applications of the *L. dispar* NPV were made with a truck-mounted mist blower. To achieve thorough coverage, each plot received about 664 l per 0.4 ha of the final spray. Applications were made from 0600 to 0900 hours. The virus was applied at hectare equivalent dosage of 1.0×10^{13} PIB's per 0.4 ha. The actual amount to be applied was calculated from the size of the ground surface canopy area to be treated. Another group of oaks was sprayed with a higher dosage of 2×10^{13} PIB's per 0.4 ha.

A sticker-spreader, NU–FILM–17®, was included in the finished spray at the rate of 28 ml per 20 l. Final spray solutions had a pH range of 4.5 to 5.5, with a mean of 5.2. Each treatment was applied three times over a 31-day period. The first treatment was sprayed on July 8, 1972, the second and third on July 25 and August 8, 1972, respectively. Control areas were treated in the same manner, except NPV was omitted.

For laboratory bioassay larvae were obtained from the stock culture of gypsy moth that was initiated and

maintained as previously reported (Yendol et al. 1973). All insects were reared on artificial diet prior to the bioassay.

The foliage samples from each cardinal direction were divided into five subsamples and bioassayed against third-instar larvae weighing 24.62 mg, ±6.64). Ten larvae were allowed to feed on the treated foliage for 5 days, and then transferred to nonsprayed foliage of the same species for an additional 9 days. Leaves were replaced with new foliage from the same source as they were consumed or wilted. Mortality was recorded at 3- to 4-day intervals during the 14-day bioassay.

With reference to the results, significant differences in the initial activity (0 hours) did not occur among the residues of nonpurified or purified virus, without adjuvants, and purified virus with IMC-90001® or Nutrilite-849® formulations (table 6.3–21). The initial activity of nonformulated NPV did not differ from that of virus residues containing india ink, molasses, egg albumin, or peptonized milk. However, at 36 hours after application, virus formulations of molasses, egg albumin, and peptonized milk retained 50 percent of their original activity, whereas in NPV without adjuvants, only 30 percent activity remained after the same time interval. The half-life of nonformulated virus was less than 12 hours. Formulation of NPV with molasses, egg albumin, and peptonized milk provided twice the mortality of virus without adjuvants at 132 hours after application. Variance between dissipation rates of purified and nonpurified virus residues was not observed.

Mean total mortalities computed individually for each replication, inclusive of all treatments where larvae were fed field-collected foliage, indicated a decrease in mortality with replication. Mortalities recorded for replications 1, 2, and 3 with foliage sampled immediately following application were 62.8, 44.4, and 31.1 percent, respectively.

Calculated median lethal times (LT_{50}) for first-treatment larvae fed NPV formulation treated foliage and sampled immediately after application (0 hours) were as follows: IMC-90001®, +14.0 days; Nutrilite-849®, +14.0 days; purified virus, 13.1 days; nonpurified virus, 12.1 days; india ink, 12.4 days;

Table 6.3-21.—*Mortality of larvae feeding on oak foliage treated with NPV formulations sampled at different postspray hours*

Formulation	Percent mean larvae[1] mortality by hours after application						
	0	12	36	60	84	108	132
Control	67.a	3.9a	3.3a	4.2a	1.2a	4.2a	3.0a
IMC-90001®	26.2b	10.8	6.8ab	6.9a	14.2abc	11.3ab	4.1a
Nutrilite-849	34.7bc	16.1ab	9.3ab	11.3abc	12.abc	14.9abc	4.9a
Purified virus	44.0bcd	19.1ab	13.2abc	9.8ab	12.1ab	7.6a	6.0ab
Nonpurified virus	46.5bcd	19.2ab	17.4abc	11.abc	8.6ab	9.3a	5.3a
India ink	47.6cd	33.5bc	20.3bc	14.8abcd	13.7abc	26.8bc	10.1abc
Molasses	51.3cd	45.7c	34.5d	24.8d	24.9bc	14.9abc	18.8bc
Egg albumin	51.7cd	39.0c	34.5cd	22.4cd	28.9c	25.8bc	13.7abc
Peptonized milk	64.0d	47.3c	35.1d	20.9bcd	21.0bc	27.7c	19.8c

[1]Means within a column not followed by the same letter are significantly different (*P*<0.01).

molasses, +14.0 days; egg albumin, 9.0 days; and peptonized milk, 9.7 days.

Increase in dose from 1×10^{13} PIB's per 0.4 ha to 2×10^{13} PIB's per 0.4 ha in formulations with india ink (5 percent v/v final spray volume (FSV) did not significantly increase mortality or extend persistence of the formulation (table 6.3–22). Reduction in the FSV of egg albumin from 5 to 1 percent (w/v) with virus applied at 1×10^{13} PIB's per 0.4 ha resulted in residues with significantly decreased activity at 36 and 60 hours after application (table 6.3–23). Mortalities at both concentrations were equivalent for the initial 12 hours.

Larvae feeding on foliage residues of egg albumin and peptonized milk in the absence of virus exhibited greater mortality than larvae feeding on other residue samples. The reason for the very high mortalities of 52.5 and 39.7 percent occurring with the egg albumin and peptonized milk without virus at 12 hours postapplication is unknown.

On the basis of the results of the 1972 ground spray and laboratory evaluations, aerial application of NPV was planned for 1974. Prior experience with timing and application equipment derived from the work with *Bt* were used in planning the test. An attempt was made to keep the population density range of the

Table 6.3-22.—*Mortality of larvae feeding on oak foliage treated with different doses of virus at a constant concentration of india ink sampled at different postspray hours[1]*

PIB's per 0.4 ha applied	Percent mean larvae mortality by hours after application[2]						
	0	12	36	60	84	108	132
2×10^{13}	51.9a	47.4a	17.6a	18.0a	6.7a	9.3a	10.7a
1×10^{13}	49.5a	31.9a	12.2a	16.1a	12.5a	16.6a	5.1a
Control	7.0b	4.3b	3.8a	2.0a	.5a	9.1a	1.5a

[1]India ink at 5 percent (v/v standard) FSV final spray volume.
[2]Means within a column not followed by the same letter are significantly different (*P*<0.01).

Table 6.3–23.—*Mortality of larvae feeding on oak foliage treated with different concentrations of egg albumin at one virus dose sampled at different postspray hours*[1]

	Percent mean larval mortality by hour after application[2]						
Final spray concentration	0	12	36	60	84	108	132
5 percent	30.2a	22.5a	18.1a	20.2a	16.3a	37.4a	12.6a
1 percent	42.9a	31.1a	13.1b	10.4b	10.1a	52.3a	11.7a
Control	4.7b	6.5b	4.7b	3.7b	2.6a	3.1b	4.2a

[1] 1×10^{13} PIB's per 0.4 ha virus dose.
[2] Means within a column not followed by the same letter are significantly different ($P < 0.01$).

test plots low to medium to avoid collapse of high-density populations (which would then destroy analysis) or to use very low populations, where foliage protection and population effects between treatment and control would be very difficult to evaluate.

Aerial Application of the Virus

1974

On May 24, application of virus was made at the rate of 1×10^{12} PIB's per 0.4 ha. The prespray egg-mass density ranged from 1,479 to 1,976 masses per 0.4 ha. The spray formulation consisted of the following materials and their rates per 0.4 ha: A commercial adjuvant-extending material, Shade® (International Mineral Corporation, Chicago, Ill.), 453.59 g; Chevron sticker-spreader®, 177.44 ml; a stabilized molasses material, CIB® (Cargill Insecticide Base), 1892.5 ml; NPV, 1×10^{12} PIB's; and water, 5.679 l. Each 0.4 ha received 7.57 l of finished spray material. The NPV application was made with a 450 hp Grumman AgCat® equipped with six Beecomist® nozzles. Application was made at a height of 9–18 m above the canopy at a speed of 2.1–2.4 km per minute.

On June 23 an estimate was made of the mean number of larvae found in the virus-treated plots and in the untreated plots. There were about twice as many larvae in the untreated area as in the virus-treated plots. Application of the virus was delayed because of weather; consequently, defoliation levels reached 50-75 percent in the treated plots. In the controls, 95–100 percent defoliation was attained. Fall egg-mass density (FEM) was significantly less in the virus-treated plots than in the controls (fig. 6.3–26). Spring egg-mass counts (SEM) were all approximately the same density. It was determined from these experiments that other virus trials should be initiated using two applications of virus applied at a lower concentration.
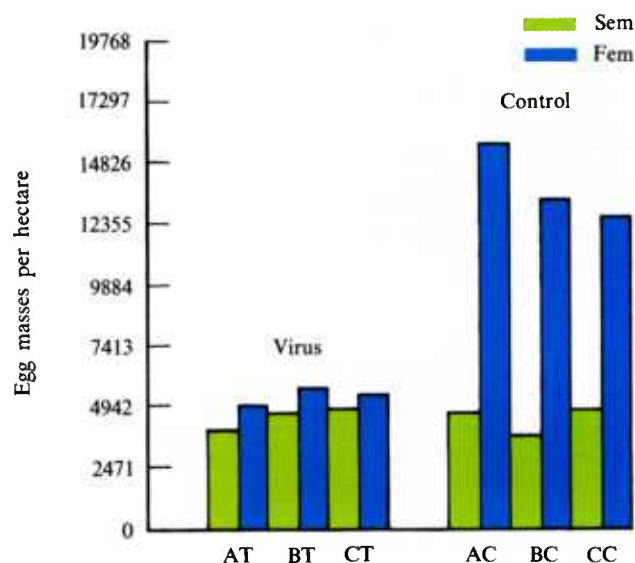
Figure 6.3-26.—*Egg-mass changes, 1974 NPV aerial spray.*

1975

The encouraging results in the 1974 aerial application test led to a larger field test in 1975 (Wollam et al. 1978).

The Hamden standard virus material cleaned with a K rotor was used and was applied twice approximately 7–10 days apart. Application rate was 18.7 l per 0.4 ha. Two formulations were used: 1.12 kg Shade®, an ultraviolet screen, 0.88 l Chevron spray sticker®, 2.81 l Cargill Insecticide Base (CIB)®, and 15.44 l of water per 0.4 ha; and 9.34 l Sandoz virus adjuvant (SVA)® and 9.34 l water per 0.4 ha. Application was made by a 450-hp Grumman AgCat® equipped with standard boom and six Beecomist® spinning nozzles with 80–100 μ perforated metal sleeves.

Three replicates (14.2 ha) were used for the four treatments, and three control blocks were established. Data were collected in ten 0.01-ha subplots in each block.

Treatments were evaluated on the basis of egg-mass numbers (pretreatment and posttreatment), number of larvae and pupae under burlap bands, defoliation estimates, spray deposit, and bioassay of leaf material collected after spray.

Virus-treated blocks had significant increases in virus incidence when compared with the control blocks, and there were significant differences in defoliation in the treated plots as compared to control plots (table 6.3–24).

Table 6.3–24.—*Degree of defoliation occurring in plots treated with gypsy moth NPV, 1975 aerial test*

Treatment	Mean percent defoliation
SVA (1 application)	69 (26)[1]
SVA (2 applications)	17 (13)
CIB (1 application)	23 (31)
CIB (2 applications)	36 (36)

NOTE: SVA = Sandoz Virus Adjuvant Formulation®;
CIB = Cargill Insecticide Base Formulation®.
[1] Standard deviation.

Spray deposit data indicated that the SVA® formulation resulted in three times the coverage than the CIB® formulation (33 drops per square centimeter versus 10.2 drops per square centimeter). The mass median diameters (mmd) for the SVA® formulations were 320 μ and 310 μ for the CIB® formulations.

Bioassay of sprayed and unsprayed foliage from the plots showed a high incidence (63–76 percent) of virus immediately after spray as determined by microscopic examination of dead larvae. This incidence dropped to about 25 percent after 3 days and continued at this level for 2 weeks or more. The second application of virus showed immediate rise in incidence of NPV to about 75 percent, with a 2-day fall back to the 25 percent level.

Egg-mass population change is shown in figure 6.3–27. Population reduction was achieved with the single and double application of SVA® but was not achieved with the CIB® formulation, principally because of the reduced amount of CIB® (12.5 percent of total formulation). The low-density plots treated in 1975 were examined in 1976 for carryover effect of the
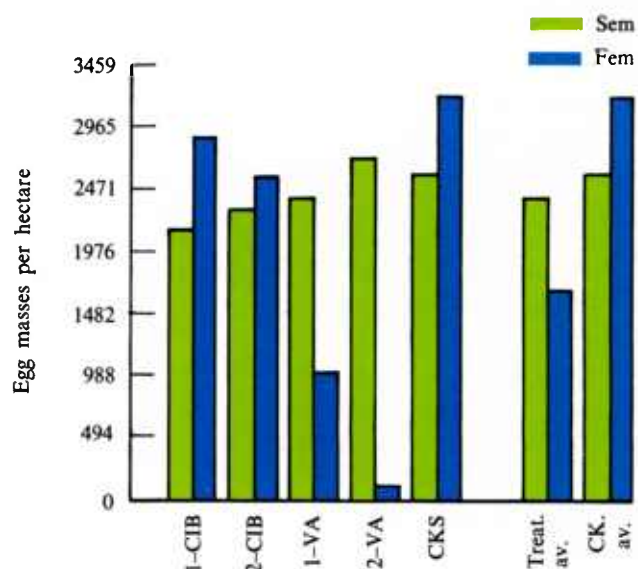
Figure 6.3–27.—*Egg-mass changes, 1975 NPV aerial spray.*

treatment. The second-year evaluation indicated that the number of eggs per egg mass did not increase after treatment as reported for *Bt* treatments (Kaya et al. 1974), or following chemical pesticide treatment (Doane 1968). Second-year defoliation in all treated plots (20 percent) was considerably reduced as compared to untreated plots (40 percent).

1976

In fall 1975, a new NPV product was developed. The major difference between this new product and the material cleaned with a K rotor used in earlier tests was that the K rotor step was eliminated and that the virus slurry did not go through the K rotor ultracentrifuge but was air dried. This change in product necessitated further efficacy evaluation. The new product (dried slurry) contains more proteinaceous material and free virions and thus can be expected to exhibit greater efficacy because of the protective characteristics of these proteins.

The 1976 field operation was designed to test lower doses of NPV, further test the Sandoz® virus adjuvant, and, because of the relatively poor performance of the molasses formulation (CIB) in 1974, test a return to 25 percent molasses from the 12.5 percent tested in 1974.

Three replicates of 14.2 ha each were treated with two doses of NPV: 1×10^{11} PIB's (25 million potency units) per hectare and 5×10^{11} PIB's (125 million potency units) per hectare. Single and double applications were made and all applications were made in the evening. Two formulations were tested: Feed-grade molasses 2 l, Chevron spray sticker® 168g, Shade® 0.45 kg, water 61; and SVA® 41, water 41. Application was made by a 450-hp Grumman AgCat® equipped with standard boom and six Beecomist® nozzles with 80-100 μ perforated metal sleeves. Application height was 15 m, swath width 22.5 m, and airspeed 150 km/h.

Table 6.3-25 presents the information on incidence of NPV, determined by microscopic examination of dead larvae, in treated and control plots. The table shows that treatments markedly increased NPV incidence, that a double application of a low dose was as good as a single application of a high dose, and that

the molasses and SVA formulations were about equivalent.

Table 6.3-26 presents data for final defoliation and egg mass change for the 1976 field test. The data also show that a double application of the low dose achieves as good foliage protection and population reduction as the double high dose regardless of formulation. The single application of the high dose provides significantly less foliage protection and population reduction than the double applications.

An aerial infrared photograph (fig. 6.3-28) illustrates the degree of foliage protection achieved by the double application low dose.

Table 6.3-25.— *Effect on treatment on the incidence of NPV in gypsy moth population 1976*

	Percent NPV[1]		
Treatment	6/8/76	6/15/76	6/25/76
1×10^{11} PIB's per 0.4 ha, twice, molasses	19.85	20.67	66.00
5×10^{11} PIB's per 0.4 ha, once, molasses	14.06	13.67	68.20
5×10^{11} PIB's per 0.4 ha, twice, molasses	13.15	54.67	51.77
5×10^{11} PIB's per 0.4 ha, twice, SVA®	33.34	55.00	57.20
Control	2.13	7.13	22.87

[1]By microscopic examination of larvae.

Table 6.3-26.— *Final defoliation and egg-mass data for gypsy moth NPV test, 1976*

	Percent final defoliation	Egg-mass counts per 0.4 ha		
Treatment		Spring	Fall	Percent change
1×10^{11} PIB's per 0.4 ha, twice, molasses	41.7	2,060	380	-82
5×10^{11} PIB's per 0.4 ha, once, molasses	59.0	2,073	897	-57
5×10^{11} PIB's per 0.4 ha, twice, molasses	46.7	1,667	331	-80
5×10^{11} PIB's per 0.4 ha, twice, SVA®	42.3	2,236	293	-87
Control	82.7	2,606	1,369	-47
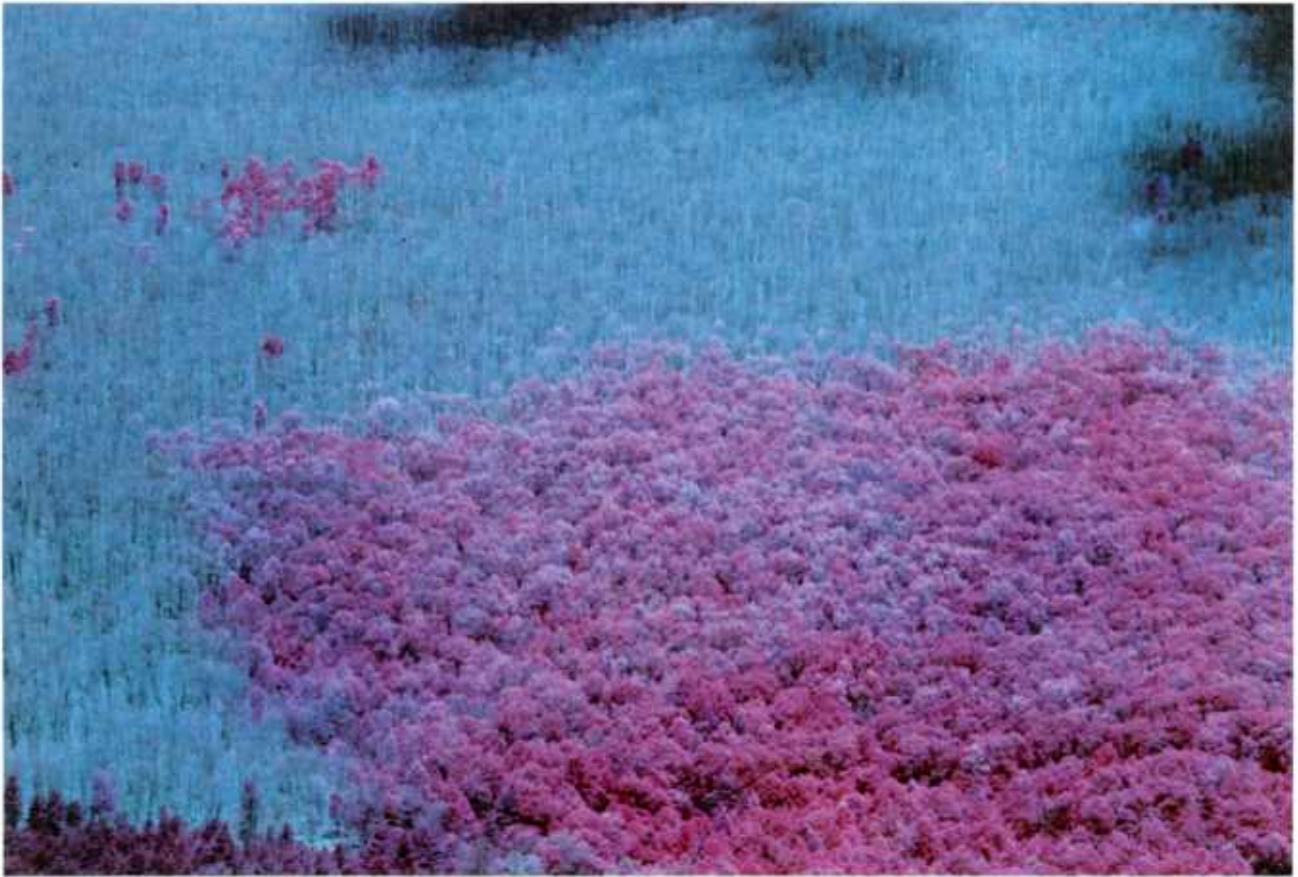
Figure 6.3–28.—*NPV plot, aerial infrared photograph, 1976.*

1977

An aerial field test was conducted with the new NPV product, Gypchek, to evaluate the substitution of spinning sleeve nozzles with flat fan nozzles, to attempt to reduce the number of applications and liters, and to compare the differences in effects between morning and evening spraying. Because of an unanticipated collapse of populations in the field, no conclusions could be drawn regarding reduced applications or morning versus evening sprays. Problems arose from the very rapid development of the larvae due to unseasonably hot weather. Rapid stripping of untreated areas around the 14.2-ha plots and degradation of the spray due to ultraviolet inactivation permitted invasion of treated plots by larvae from the outside, thus obliterating the effect of the treatments.

The successful portion of this test was carried out in central Pennsylvania on 14.2-ha plots replicated three times. One dose of NPV (1×10^{11} PIB's per 0.4 ha, 25 million potency units) was applied to all treatment plots. A single formulation (feed-grade molasses 2 l, Shade® 4 l, Chevron spray sticker ® 168 g, water 6l) was used. Application rate was 8 l per 0.4 ha for all treatments. A 450-hp Grumman AgCat® with standard boom and six Beecomist® nozzles or flat fan nozzles Spraying System Tee-Jet® (8004, 8006) were used. Height of application was 15 m, swath width was 22.5 m, and air speed was 150 km/h.

Table 6.3–27 presents the data accumulated in this test.

Table 6.3-27.—*Final defoliation and egg mass data for gypsy moth NPV test, 1977*

| Treatment | Percent final defoliation | Egg-mass counts per 0.4 ha | | |
		Spring	Fall	Percent change
1×10^{11} PIB's per 0.4 ha twice Beecomist®	49	1,337	303	-77
1×10^{11} PIB's per 0.4 ha twice flat fan	55	1,184	410	-64
Controls	80	1,154	2,293	+37

Conclusions drawn from this test indicate that no significant (5 percent level) difference in efficacy occurs because of the type of nozzles used. The evening application of the NPV twice gave comparable results to the data accumulated in the 1976 test. Droplet sizes were 300–320 μ for both nozzle systems.

Figure 6.3–29, an infrared aerial photograph, shows the level of foliage protection achieved in the 1977 test.

Unique Applications of Gypsy Moth NPV

Although the major thrust in evaluating the gypsy moth NPV as a control tool has been through the broadcast use of the material, other less conventional techniques have been used successfully with other NPV's, including the incorporation of the virus into baits, the topical application of an NPV suspension to hatchable egg masses, the use of selected parasites as vectors of the disease, and population quality modification by introduction of very low dosages of NPV (Lewis 1975).

In laboratory evaluations, topical application of NPV to egg masses has resulted in high mortality by virus of newly hatched larvae. This technique of NPV use has been reported to be successful in tests in central Europe and in Russia.

In the laboratory, the application of gypsy moth NPV resulted in high second- and third-instar virus kill. Early results from a small field test indicate that this technique has considerable promise for spot

introduction of the virus in small isolated infestations. Further field evaluations are required to fully develop the potential of this technique for use by homeowners and in high risk infestations.

Very preliminary tests have been conducted to evaluate the incorporation of NPV in baits of artificial diet and sugary substances. In the laboratory, this technique results in high mortality by virus. Further evaluation of the potential of this technique requires more intensive field research and development.

An additional technique for use of NPV's that has been reported to be successful in some situations is the sequential (intergeneration and intrageneration) application of low dosages of NPV. The intent of this approach is to change the population quality by causing sublethal infection and to assure saturation of the environment with the NPV. A test utilizing this concept is being conducted in New Jersey with the cooperation of the State Department of Agriculture. A rapidly cycling population has been treated aerially 3 years in succession. No definite information has been obtained because the treated and the control population have remained at low levels. Followup evaluations will determine if population frequency and amplitude can be modified by this approach.

The use of parasites as vectors of NPV and *Bt* is covered in the sections on parasites (chapter 6.1) and predators (chapter 4).

Natural Virus Detection

Normand R. Dubois

The early detection (microscopic or submicroscopic) of NPV infection in pest insects has long been considered a desirable analytical tool. Development of such a technique for gypsy moth larvae could be used to determine the general health of a field population, assess the efficacy of application of NPV for control of the pest, and provide an early assessment of the extent of natural virus load in a population when determining the necessity of applying control measures to an infestation.

During the process of infection, viral genome-mediated protein synthesis initially takes place in

susceptible host tissue cells (hemocytes, fat body cells, and dermal cells). Also, in the hemolymph, synthesized viral proteins accumulate concurrent with the development of an altered larval protein pattern (Young and Johnson 1972, Young et al. 1975). These deviations from the normal protein pattern of uninfected larvae should be observable shortly after the onset of infection. Both electrophoretic and immunodiffusion techniques have been used to detect the presence of viral proteins in insects. However, neither method has thus far been successfully used in early detection of virus infection. Variabilities between individual larvae, lack of resolution and difficulty in separating specific proteins (electrophoresis), or difficulty in preparing specific viral protein

antibodies (immunodiffusion) may be the limiting factors.

In 1976, studies on early detection of NPV infection in second- and third-instar gypsy moth larvae were initated using isoelectric focusing (IF) of hemolymph protein; presence of alkaline protease in the subdermal fat body tissue; and refractometric analysis of the hemolymph.

Using IF techniques, a unique protein with an isoelectric point at pH 3.95 appears in the hemolymph 6 to 7 days postingestion of NPV. This unique protein persists and is also found in larvae in advanced stages of NPV infection. Using this protein as a marker for infection, quantitative estimates on aliquot populations of NPV-infected larvae could be predicted in

Figure 6.3–29.—*NPV plot, aerial infrared photograph, 1977.*

larval populations exposed to low and medium doses of NPV as well as in untreated controls with reasonable accuracy when actual mortality was measured 17 to 21 days postexposure to NPV. But when the larval populations had been exposed to LD_{90} doses, the prediction grossly underestimated the actual mortality, which occurred 10–14 days postexposure to the virus.

Similar results were obtained when the presence of alkaline protease activity in fat body tissue was used as the marker, except that prediction could be made 5 days postexposure to the NPV and the entire analysis completed in 1 to 4 hours compared to 3 hours of focusing and overnight destaining when using the IF procedure.

Also, reagents and equipment used for detecting the presence of alkaline protease activity were far less expensive than those required for IF, a point of considerable importance when contemplating the testing of large numbers of larvae. This procedure was also field tested in two virus-sprayed plots and one untreated control plot. The results were most encouraging—the use of the technique indicated that no significant virus mortality was to occur in any of the plots and none did. However, because of the limited sampling, these studies will have to be repeated and the results verified.

Finally, when refractometric measurements of the hemolymph were used to predict infection, it was found that quantitative predictions of percent virus mortality could be made with good accuracy 3 to 5 days postexposure to low or medium doses of NPV and controls (actual mortality occuring 21 days postexposure). Further, as with the other two procedures, quantitative predictions of virus mortality in populations exposed to LD_{90} doses of NPV resulted in gross underestimation of the actual mortality. This method is preferred to the other two in that it is the simplest of the three, it is the easiest to use, and the results are known immediately. However, measurements cannot be accurately made on fourth- or fifth-instar larvae or just prior to or just after molt.

It should be noted here that all three procedures are based on a sequence of physiological responses that take place when larvae are challenged by virus. At high virus titer, not only do all three methods grossly

underestimate the actual mortality, but the incubation period for virus mortality is shortened by one-third. These observations seem to suggest that the mechanism of virus invasion and route of infection may differ depending on the virus titer in the host at the initiation of infection—in which case different techniques may possibly have to be devised to predict mortality accurately in larvae exposed to high doses of NPV, compared to controls and low- and medium-dose exposures.

Insect populations such as the gypsy moth are often prone to virus epizootics. Conditions leading to outbreaks of disease that result in drastic reduction of the pest populations are not fully understood and are therefore unpredictable. This sometimes results in the implementation of unnecessary control measures on larval populations that would otherwise be reduced naturally below pest levels. The procedures previously described are but a first step in the development of a procedure usuable for predicting virus diseases in natural populations. At present these techniques provide only an immediate estimate just prior to actual application, but with refinement they could be improved to allow evaluation of the health status of the population immediately at larval hatch and thus provide more time for decisionmaking and arrangement of spray application schedules.

The use of these tools in conjunction with other population quality measurements may very well enable the user to estimate more accurately the need for control measures and predict the outcome of selected control tactics, including the use of gypsy moth NPV.

Registration
Franklin B. Lewis

The gypsy moth NPV (Gypchek), Environmental Protection Agency registration number, EPA–27586–2, was officially approved April 13, 1978. Registration has been a long process, aided principally by the prior registration of the Heliothis NPV (Elcar®) and to some extent by the registration of the Douglas-fir tussock moth NPV. The use pattern of the gypsy moth NPV, anticipated in generally populated areas and at frequent time intervals, is different from either

of the two previously registered NPV's. The projected use pattern plus changing scientific knowledge caused testing modifications and extensions to be imposed on the gypsy moth NPV registration package.

The principal difficulties encountered in the registration process involved the establishment of protocols and tests necessary to provide viral identity data, virus safety data for other animals, and the proper evaluation of infectivity as contrasted to toxicity evaluations required of chemical pesticides. The establishment of guidelines was accomplished by the publication of Summers et al. (1975).

Registration requires the development of data in three general areas: Safety, efficacy, and production (see the Safety, Efficacy, and Production and Quality Control sections). It is important to bear in mind that it is not the virus that is registered but a final product, based on a defined production procedure, containing the virus as an active ingredient.

Registration requirements for NPV's are still somewhat in a state of flux and can be modified by the anticipated use pattern of the material, new analytical procedure development (such as restriction endonuclease studies for viral identity), or new application technique developments.

For a material to be used as an insect control agent, it must be registered for that use. The material cannot be used in any form or manner other than that specified on the registered label. (See figure 6.3–30, an illustration of the present label for aerial application of the gypsy moth NPV.)

Cost Effectiveness
Franklin B. Lewis

A discussion of the cost-effectiveness relationships involved in the use of the gypsy moth NPV is not a clear-cut summation of the cost of the material, its formulation and application costs, and the expected dollar benefits of its use. Environmental and ecological aspects of its use must be considered, and the desires, restrictions, and plans of the land manager also play an important role in the estimation of cost benefits.

Perhaps the best way to evaluate cost effectiveness would be to discuss the definite plus characteristics of

gypsy moth NPV, the definite negative characteristics and finally the characteristics that may be either positive or negative depending upon the user's objective and expectations.

From the positive side, gyspy moth NPV has no demonstrable effects on beneficial forms of life, it is a natural component of the gypsy moth ecosystem, it does not adversely affect other natural mortality agents, it affords population reduction and foliage protection, and it is a sound environmental and ecological pest management tool because of its safety and compatibility with other forms of insect control. There is information, as yet not fully documented, that NPV exhibits carryover effects into the next generation. There is a final positive element that holds for NPV as well as other microbial agents: The lack of demonstrated development of insect resistance to the use of NPV's. This is in marked contrast to most of the synthetic pesticides and is an important consideration when contemplating treatment of the same populations over time.

From the negative side, NPV's are relatively slow acting, taking about 10–14 days for infection to develop and feeding to cease, and they require considerable care in their application and in the timing of application and may require more than one application. At present, gypsy moth NPV is more expensive than conventional pesticides.

One principal attribute of gypsy moth NPV exists that can be considered either negatively or positively: The NPV is selective against gypsy moth larvae. This can be a positive attribute if a user is confronted with only a gypsy moth problem; however, if a serious mixed infestation of pest insects is involved, only gypsy moths will be killed, leaving other pests untouched. Another attribute of this NPV with positive or negative implications is the rapidity of loss of virulence in broadcast application. For a user who desires or needs short residual this is a positive aspect; however, extended hatch and development of the insect, which create the need for longer residual activity, would be a negative attribute.

Thus, cost effectiveness falls on the benefits side if environmental considerations are strong, if minimal damage to other natural factors is desired, if the infested area is mainly infested with the gypsy moth,

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PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS

WARNING
Causes eye irritation. Do not get in eyes.

FIRST AID

In case of eye contact,immediately flush
eyes with plenty of water for at least 15
minutes.For eyes,call a physician.

ENVIRONMENTAL HAZARDS

Avoid application to lakes,streams,or ponds.
Do not contaminate water by cleaning of
equipment or disposal of wastes.

DIRECTIONS FOR USE
GENERAL CLASSIFICATION

It is a violation of Federal law to use
this product in a manner inconsistent with
its labeling.

For foliar protection from gypsy moth larvae
make 2 applications 7 to 10 days apart at the
rate of 25.0 to 125.0 million gypsy moth poten-
cy units per acre in sufficient water for thor-
ough and uniform coverage. Stickers and u.v.
protectants may enhance performance of this
product. Refer to technical bulletin for mix-
ing and application instructions.

NEVER USE CHLORINATED WATER IN THE SPRAY
FORMULATION.

STORAGE AND DISPOSAL

Activity may be impaired by storage above 90°F.

Do not contaminate water,food or feed by
storage or disposal. Open dumping is prohibited.
Do not reuse empty container.

Pesticide, spray mixture, or rinsate that cannot
be used should be disposed of in a landfill
approved for pesticides or buried in a safe place
away from water.

Container disposal: Triple rinse and dispose
of in an approved landfill or bury in a safe
place.

Consult Federal,State, or local disposal
authorities for approved alternative procedures.

GYPCHEK

**BIOLOGICAL INSECTICIDE
FOR THE
GYPSY MOTH**

Active Ingredient*:
(Polyhedral inclusion bodies of gypsy moth
nucleopolyhedrosis virus). 4%

Inert Ingredients. 96%

Total   100%

*This lot contains at least ___ million
gypsy moth potency units per gram.

KEEP OUT OF REACH OF CHILDREN

WARNING

See back of tag for additional precau-
tionary statements.

For use by or under the supervision of U.S.
Forest Service.

Notice: The U.S. Forest Service makes no
          warranty, expressed or implied
          including the warranties or mer-
          chantability and/or fitness for
          any particular purpose, concerning
          this material except those which
          are contained on the U.S. Forest
          Service's label.

MFG. BY: U.S. Forest Service, USDA
           P.O. Box 2417
           Washington, D.C. 20013

EPA ESTABLISHMENT NO.27586-CT1

EPA REGISTRATION NO. 27586-2

Net Weight _____   Lot No. _____

Figure 6.3-30.—*Illustration of the present label for
aerial application of gypsy moth NPV.*

and if careful timing and application are not constraints. Cost effectiveness decreases if environmental considerations are low, infestations are mixes of pests capable of causing significant damage, immediate kill is required, and long residual activity is necessary.

The long-term effect of NPV is still being evaluated and positive findings could significantly tilt cost effectiveness to the benefit side if 1-year use can exert desired effects in subsequent generations.

Although the gypsy moth NPV is somewhat more expensive than conventional pesticides, cost has been significantly reduced and can be reduced still further. At the beginning of the expanded program, the cost of using the NPV as a broadcast spray was about $75 per treatment per hectare. The cost has now been reduced to between $15 and $20 per treatment per hectare. Extrapolation of present formulations and NPV production provides an estimate of about $7.50 to $8.75 per treatment exclusive of application costs. The cost of $2.50–$3.75 for the NPV product is well within reach.

Cost reductions in the use of the NPV product can be realized by optimizing production technology and using automation where possible; improving formulations to extend activity and eliminate the need for double applications; developing more efficient, effective methods of disseminating the NPV to reduce the wasteful practice of broadcast spraying; and developing mass production systems based on tissue culture to reduce quality control costs and take advantage of modern viral production technology.

As can be seen, costs of utilizing gypsy moth NPV can be radically improved with a strong research effort.

Summary
Franklin B. Lewis

Short-Range Needs

There are two areas requiring more research and development work in the short term (1–3 years). One of these is formulation and application. Improvements in formulations to extend the virulence of the gypsy moth NPV are critically needed to optimize the broadcast effects of this material. This would include work on ultraviolet screening compounds; replacement of the water carrier with nonphytotoxic, less evaporative material; droplet data development with regard to coverage, sizing, and life span; vertical and horizontal distribution in broadleaf canopies; and field dose mortality data expansion. Concurrent with formulation improvement and optimization, development and modification of application techniques should be investigated. Since broadcast dissemination (the standard registered method in forest situations) of the gypsy moth NPV by air or on the ground is essentially a wasteful, inefficient technique, more attention should be placed on alternate application techniques such as baiting, trapping, and development of refined and imaginative techniques for aerial application.

In addition to improved formulation and application techniques, studies should be developed to evaluate the role of the NPV in integrated pest management schemes. This should be done by both computer simulation and empirical field studies.

It is the universal opinion of workers involved with the development of NPV's that the most important area for improvement is in formulation and application. This is equally true for other microbial agents such as Bt, fungi, other viruses, and protozoa.

Another area of considerable interest and need is more basic and involves the mode of virus replication, mode and means of spatial and temporal transmission, and detection and prediction of NPV. These data are extremely pertinent to gypsy moth control decisions, population prediction, and estimates of ultimate damage potential.

Little is known about the natural transmission of gypsy moth NPV, by what method and in what form the virus (if it does) passes from one larva to another in a given generation. Also, the manner and form of transmission from generation to generation are essentially unknown. How the virus persisting in the environment might infect future generations of the

insect is imperfectly understood. Specific details of the rate of intracellular infection and the form of the infectious unit are lacking.

All these details are necessary for the construction of an epizootiological model that bears a reasonable resemblance to the real-life situation. In turn, the development of this disease model is essential to practical prediction of the population fluctuations of the insect.

Not only do we have an imperfect understanding of the behavior and activity of this disease under natural conditions, but we also do not have a reasonable knowledge of the impact or modication of the natural disease when we apply the disease agent to these populations by artificial means.

A final and very important research and development need is to improve production of the NPV to further reduce costs, to improve stability and effectiveness of the material, and to refine the product to eliminate the present imperfections due to production technology.

Although the present production technique is an in vivo one, work should continue to evaluate and develop alternate methods of production such as tissue culture.

Therefore, there are three important research and development needs in the short term: the improvement and optimization of formulation and application; better understanding of the epizootiology of the disease, both natural and artificially induced; and improvements and refinements in NPV production technology to improve stability and efficacy and to reduce production costs.

Long-Range Needs

Several of the points discussed under short-term needs actually carry over to long-range needs (3–5 years).

One of these areas is in optimizing NPV production. Real advances in cost reduction and efficacy improvement of in vivo production lie in the area of automation of the system and full development of the laboratory rearing of diapause-free insects. Full development and implementation of an optimal system based on the above factors will take several years of carefully planned research and development.

However well an in vivo system is developed, it will have inherent defects that cannot be overcome, such as inevitable contaminants, biological variability, and critical steps involving manual labor. The most viable alternative to the in vivo system is the development of an in vitro system. Such a system based on the culture of insect cells will eliminate most of the difficulties associated with the larval rearing system and quality-control procedures. But a lengthy research and development program would be required to develop an in vitro system of mass producing the gypsy moth NPV economically and efficiently.

Formulation and application improvement is another area that can be a long-range need. Quick empirical approaches to this problem have been applied to this critical problem but have usually not resulted in the desired effect. A systematic planned approach with proper attention to the biological, physical, meteorological, and economic aspects of the problem will take several years, but ultimately will result in the desired effect.

The multigeneration effect of the use of the gypsy moth NPV is both a short- and long-term need, particularly in the concept of population stabilization. A corollary to this is the need to evaluate the integration of the NPV with other control tools to bring about population stabilization or continued reduction below desired economic thresholds.

A final long-term need is to determine if latency of NPV occurs in the gypsy moth. Latency, or the existence of the virus in a hidden or nonactive state, would have significant impact on the epizootiology of the virus naturally or artificially applied. Disease models and population predictions would have to be modified from existing concepts, and external population influences (moisture, nutrition, etc.) would have to be reevaluated. Insect virus latency is controversial, and proof of its existence in the gypsy moth is lacking. A long-range research effort will be needed to elucidate this.

Future Forest Insect Virus Use

The gypsy moth NPV product is the first registered by the Environmental Protection Agency for use on deciduous hardwood trees in the populated Eastern United States. This means that these kinds of materials may be approved in the future for this use pattern.

The implication of this approval is that more environmentally acceptable control alternatives may be made available for use alone and in conjunction with other tools in pest management systems. The overall aim of reducing pesticide loads in the environment, therefore, becomes more possible, and another class of control tools can be utilized in a forest pest management scheme.

An important aspect of the approval of gypsy moth NPV is that further research and development is required. Much remains to be done in optimizing many aspects of NPV use as its now exists.

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6.4 Pheromones

Introduction

E. Alan Cameron

For more than eight decades, entomologists have recognized that the adult female gypsy moth emits an odor that is attractive to male moths. Fernald (Forbush and Fernald 1896) noted that, after emerging from the pupal case, "... by the time the wings have expanded, and sometimes before, the female begins to attract or "assemble" the imagoes of the opposite sex," and that it was "a well-known fact" that it was the unfertilized females that were attractive. He and his coworkers attempted to exploit this phenomenon to trap enough males in an infestation so that few would be left to inseminate the females. With a high proportion of unfertilized eggs then laid, it was expected that the population in that locality would be reduced the next year. Although they caught thousands of male moths, very few egg masses collected subsequently from areas where trapping had been conducted were infertile. Their elusive dream has been pursued by a succession of workers through the years using female moths, extracts, or synthetic lures as baits in traps (Collins and Potts 1932, Maksimović 1959, Knipling and McGuire (theoretical discussion) 1966, Beroza and Knipling (theoretical discussion) 1972, Beroza et al. 1973, E. A. Cameron 1973). These attempts at gypsy moth population manipulation have comprised only one component of the broad-ranging and complex studies of the chemical ecology of the gypsy moth.

As early as 1913, efforts to identify the chemical nature of the attractive material(s) emitted by female moths were initiated under the sponsorship of the U.S. Department of Agriculture (Collins and Potts 1932). Acree (1953a) gave the name "gyptol" to the active fraction of moth extracts, and this material was later identified as (+)-10-acetoxy-1-hydroxy-cis-7-hexadecene (Jacobson et al. 1960). Subsequent field tests of gyptol and a synthetic homologue, gyplure, gave erratic and nonpredictable results and culminated with a direct challenge to the validity of the chemical determination by Eiter et al. (1967). This identification was retracted following reinvestigation by Jacboson et al. (1970). Bierl et al. (1970) followed

very shortly thereafter with a paper claiming cis-7, 8-epoxy-2-methyloctadecane ("disparlure") to be the attractant. More recently, the chiral nature of the disparlure molecule has been intensively investigated, and Iwaki et al. (1974) were the first to recognize that the (+) enantiomer is much more attractive to male moths than either the (-) enantiomer or a racemic mixture of the two.

While the isolation, identification, and synthesis of the chemical attractant of the gypsy moth were being pursued, female moths, extracts from females, and other synthetic materials have been used as baits in traps of various kinds (Beroza 1971). These traps were widely deployed throughout the United States and parts of Canada to monitor the spread of this exotic pest through the capture of male moths. Both the form of the trap and the method of presenting the bait have been changed many times over the years (see chapter 6.4, Disparlure-Baited Traps for Survey and Detection) and refinements will continue to occur as our knowledge increases. But this role of survey and detection is an exceedingly important one and one that has employed an attractant in successful operational use for many years.

Survey and detection are examples of qualitative use of the attractant. It is one thing to catch a male moth or moths in a trap but quite another to know what that catch means in terms of the gypsy moth population in the area. Pest management is directed against populations, not individuals. If there was some way of measuring or, even more desirable, of predicting population levels based on capture of male moths in traps, we would have a very valuable tool indeed. Granett (1973) designed a high-capacity box trap that he suggested (1976) might have value in assessing insect numbers even in dense populations. Mastro et al. (1977), using behavioral observations, assessed the efficiency of various kinds of traps—information that is vital to have if traps are to be used in a quantitative manner.

Over the years, and particularly during the 1970's, much time and money have been expended in evaluation of broadcast applications of disparlure for direct manipulation of field populations of the gypsy moth. Permeation of the atmosphere with enough of

the material to create disruption of the chemical communication between the sexes has been the major goal. If success could be achieved, the attractant might well then find an important role as one of the available tools for managing the gypsy moth. Most scientists agree that the use of pheromones for manipulating insect populations shows an inverse density-dependent relationship—that is, the pheromone will be more effective as populations are reduced to lower and lower levels but will be less effective at higher insect population densities. Given this mode of action, there are particular situations in which a tool such as broadcast application of a pheromone could be exceedingly valuable.

Each year survey and detection efforts (which rely now on the use of pheromone-baited traps) locate male gypsy moths in areas remote from known infestations. Sometimes these captures are solitary moths spread presumably through man's activities and are not at all indicative of an infestation; at other times subsequent scouting may reveal an isolated or incipient infestation. Elimination or eradication of such an infestation is often desirable and would seem to be a prudent goal. A number of tests, in both simulated and actual field situations, have been conducted to test the principles involved in meeting the challenge (Stevens and Beroza 1972, Beroza et al. 1973, E. A. Cameron 1973, Cameron et al. 1974, Schwalbe et al. 1974, Cameron 1976). Cameron et al. (1975) also tested the olefin precursor of disparlure for the same purpose. Thus far, no tests using either simulated or wild populations and carried out under natural conditions with feral (as opposed to laboratory-reared) insects have been successful in the sense that all mating was prevented.

Another set of circumstances in which sparse populations of the gypsy moth occur is along the so-called "leading edge" of the infestation as it advances south, west, and north from the generally infested Northeastern United States. Both Beroza and Knipling (1972) and Beroza et al. (1974b) suggested the possibility of establishing a barrier zone to contain the moth within the infested Northeastern States, and Beroza et al. (1974b) even suggested that "progressive movement of the barrier northward to shrink the infested area eventually may be possible . . . ." Granett (1976) addressed this issue, pointing out potential environmental degradation as a result of large-scale aerial application of chemical pesticides that would be required under a barrier zone approach, the costs involved, and concern over the political feasibility of such an undertaking.

Various other strategies have been considered to retard the spread of the insect, and some are discussed elsewhere in this book. Again, because of the inverse density-dependent nature of the pheromone, it has been considered to be a candidate material for incorporation into a containment strategy. The principles involved and the experimental evidence upon which a decision for use would be predicated are essentially the same as those associated with eradication. In this case, however, the pheromone probably would be used in combination with one or more other population suppression agents such as chemical pesticides (Beroza et al. 1974b). Both Beroza et al. (1975a) and Cameron and Mastro (1976) have evaluated field tests in which disparlure in a microencapsulated formulation was applied just prior to male moth flight and after two (Beroza et al. 1975a) or one (Cameron and Mastro 1976) application of Sevin 4-Oil® during larval development. Neither team was able to demonstrate increased population reduction as a result of the pesticide plus the lure treatment when compared with pesticidal treatment alone. Although they worked within a larger generally infested area and not at the advancing front of the infestation, both of these teams considered that their tests were adequately buffered from the general infestation. Good data of a similar nature are not available from tests conducted on the leading edge of the infestation, in part, at least, because of the inherent difficulties of obtaining any reliable information from exceedingly sparse populations. In addition, it is extremely difficult if not impossible to locate a suitable untreated control area with which to compare results.

Any use of an insect pheromone is ultimately dependent on one factor: Alteration of normal behavior of the insect under investigation. Behavior may be altered by attracting, in this case, male

gypsy moths to a baited trap, by stimulating them to search for what they perceive as a female moth in the area, by disrupting the normal chemical communication between the sexes, or perhaps in other ways. If entomologists are going to alter behavior, it behooves them to know as much as possible about the normal, unaltered behavior of the insect in question. In the case of the gypsy moth, we are continually reminded of how little we knew when we started testing disparlure, and indeed how many gaps in our knowledge still remain. Doane (1976) noted that studies of behavior have tended to follow, rather than precede, development of pheromones for control. And Wellington (1976) made the more general observation that too often we tend ". . . to ignore insect behavior until it gets in the way of field work."

A strong case can be made in support of the statement that the most significant gypsy moth pheromone work done in the last decade has been in the area of adult behavior. By current standards, our knowledge as summarized by Doane (1968) was primitive at the time disparlure was first identified (Bierl et al. 1970). In part in response to the insistence of E. A. Cameron (1973) that behavioral studies must receive high priority, major contributions have been made by several groups, among them Doane (1976), Richerson et al. (1976a,b), Richerson (1977), and Cardé (chapter 6.4, Precopulatory Sexual Behavior of the Adult Gypsy Moth). Along the way, Richerson and Cameron (1974) demonstrated unequivocal differences in pheromone release and sexual behavior between laboratory-reared and wild gypsy moth adults, results that had major implications for evaluation of disparlure for behavioral modification and especially in laboratory tests run out of season. Other excursions have been made into investigating diel periodicity of adult behavior (Cardé et al. 1974), the spectral sensitivity of the compound eye and consequent behavioral implications (Brown and Cameron 1977), and the effect of high frequency sound on male behavior (Baker and Cardé 1978). With this solid base, we are in a much better position to pursue studies of behavior and how that behavior may be altered deliberately by

the controlled application of pheromone in whatever context.

In the following pages, many of those most intimately involved in the broader study of the chemical ecology of the gypsy moth describe the considerable progress that has been made and, in particular, the results of studies conducted under the Expanded Gypsy Moth Program. This is an unfinished story, one that will lead the reader through elegant experiments, down blind alleys, past conflicting interpretations of results, and eventually to an assessment of future needs if the gypsy moth and its chemical ecology are to be understood in the way needed if populations of this pest are to be manipulated successfully.

Chemistry and Formulation of the Attractant

Chemistry of Isolation, Identification, and Synthesis

May N. Inscoe and Jack R. Plimmer

When it was demonstrated that extracts of female gypsy moths could be used to lure male moths to traps, the isolation and identification of the attractive substance became major research goals. For many years, however, progress was extremely slow and sporadic, with many frustrations, numerous false leads, and contradictory results. Success was not achieved until a convenient laboratory bioassay had been developed, numerous microchemical techniques had been devised, and new chromatographic and spectrometric instrumentation adequate for working with minute amounts of material was perfected and became available.

Collins and Potts (1932) reported on studies undertaken by the U.S. Department of Agriculture, Bureau of Entomology, beginning in 1913, in which arrangements were made for personnel from the Harvard Medical School and later from Harvard University to investigate the chemical nature of the attractant. By 1920 it was established that the attractive material was localized on the last segment of the female abdomen and that it could be extracted by

ethanol or ether; it was less readily soluble in petroleum ether and was only slightly soluble in water.

Saponification did not destroy activity but did shorten the duration of attraction in the field; this was attributed to removal of fatty substances that impeded volatilization in the untreated extract. Some tests suggested that the material was an aldehyde, but studies in 1921 led to the conclusion that it was not an aldehyde, an acid, or a base and that it was a relatively stable saturated compound.

Activity was destroyed by cold, concentrated hydrochloric acid or by boiling alcoholic potassium hydroxide. One test suggested that the long activity of extracts resulted from continuous generation of attractive material by hydrolysis of a more complex substance.

Studies on suitable extraction solvents made between 1920 and 1929 showed that benzene, xylene, or gasoline yielded the most effective extracts. The rate of volatilization of the solvent influenced the duration of activity; extracts made with the more volatile solvents, such as ether or petroleum ether, were attractive for only 2 weeks, while with extracts in benzene or xylene, males were attracted for as long as 4 weeks. However, addition of materials such as gasoline or vegetable oils to reduce the volatilization of the attractant substance was not effective in prolonging its activity (Collins and Potts 1932).

Similar studies were also undertaken in other countries. Prüffer (1937), reporting on studies in Poland, indicated that alcoholic extracts prepared from 100, 50, or 20 female abdominal tips attracted approximately equal numbers of male moths, but those from 10 or 2 tips were less effective. Von Zehmen (1942) described tests in Austria that used extracts made with several common solvents and released insects rather than natural infestations. Most extracts, other than those made with carbon tetrachloride, were effective.

Chemical studies on the attractant were renewed by the U.S. Department of Agriculture in 1941 (Haller et al. 1944) and confirmed most of the results reported by Collins and Potts. In addition, it was found that catalytic hydrogenation of the unsaponifiable fraction produced a marked increase in attractancy. Haller et al. (1944) also reported that the active material reacted with phthalic anhydride and that it could be recovered from the phthalic acid ester by hydrolysis, thus suggesting that it was an alcohol.

Acree (1953a, b, 1954) continued these efforts to isolate the attractive substance, for which he suggested the name "gyptol." Chromatographic methods for separation of the active material were worked out, on the assumption that the substance was an alcohol. However, results with "esters" were conflicting, and Acree came to the conclusion that activity could be attributed to two or more esters from two different alcohols. This, of course, conflicts with the earlier finding that saponification did not affect activity (Collins and Potts 1932).

The observation by Haller et al. (1944) that hydrogenation increased the attractancy of tip extracts was pursued, and it was demonstrated (Acree et al. 1959) that the hydrogenation procedure was effective in stabilizing the extract, so that a 10-year-old hydrogenated extract was still attractive in the field, whereas an extract without this treatment deteriorated rapidly and lost most of its original potency before the next flight season.

About this time, Stefanovic and Grujic (1959) and Stefanovic et al. (1959) reported on studies on fractionation of extracts in Jugoslavia. After numerous steps, including hydrogenation, saponification, and steam distillation, several active fractions with a characteristic odor were obtained.

Progress on the isolation of the attractant substance had been very slow up to this time, largely because the only means of testing for attractancy was by comparison of trap catches obtained in the field with the various fractions of extract or candidate chemicals. This testing could be done only during the few weeks of the flight season each summer. A laboratory bioassay was developed (Block 1960) in which the relative number of pinioned male moths responding sexually to a stimulus and the intensity of their response were used as criteria; this bioassay and the use of laboratory-reared moths permitted year-round testing for activity with more rapid and

reproducible results and with much less material. It was recognized that the basic assumption that the materials eliciting the copulatory response in the laboratory would also be attractive in the field always required experimental validation.

This laboratory bioassay was used in the isolation of two active materials from the benzene extract of the abdominal tips from 500,000 virgin female gypsy moths (Jacobson et al. 1960, 1961). The more active of these, characterized as (Z)(+)-10-(acetyloxy)-7-hexadecen-1-ol, was designated "gyptol." The other substance was not investigated further. A synthetic homologue of gyptol, (Z)-12-(acetyloxy)-9-octadecen-1-ol, prepared as a model compound for microoxidation studies, was also found to be attractive to gypsy moth males (Jacobson 1960, Jacobson and Jones 1962). This material, called gyplure, was synthesized from the readily available ricinoleic acid and was thus considerably less costly than gyptol; it was hoped that it would find application in control measures as well as in detection and survey traps.

(Current nomenclature has been used for the chemical names of gyptol and gyplure. The name originally used for gyptol by Jacobson et al. (1960) was (+)-10-acetoxy-1-hydroxy-cis-7-hexadecene; in 1962 it was called (+)-10-acetoxy-cis-7-hexadecen-1-ol. The name used for gyplure by Jacobson and Jones (1962) was (+)-12-acetoxy-cis-octadecen-1-ol.)

In subsequent field tests, however, synthetic samples of gyplure and gyptol prepared by several different routes were inactive (Stefanovic et al. 1963, 1965, 1969, Burgess 1964, Eiter et al. 1967), and the original materials were reinvestigated (Jacobson et al. 1970). The unpublished observations of Collier (1962, cited in Jacobson et al. 1970) were confirmed. A less polar, biologically active component could be separated from active samples of gyplure by thin-layer chromatography (TLC), and the purified gyplure recovered from the TLC plates was inactive. Likewise, synthetic gyptol prepared by three synthetic routes was inactive in field and laboratory bioassays, and gas chromatographic analysis of the original active sample of gyptol (Jacobson et al. 1960) showed that an attractive substance with a much shorter retention time than gyptol was present in exceedingly small quantities. Gyptol was present in relatively large amounts in the extract from virgin female moths, but, like gyplure, it was not attractive to male moths and was not the sought-after gypsy moth sex attractant.

Investigation on the nature of the attractant continued; the pheromone was isolated and its identification as cis-7,8-epoxy-2-methyloctadecane was announced in 1970 (Bierl et al. 1970, 1972). The name "disparlure" was given to the material. In these studies an extract of abdominal tips from 78,000 virgin female moths collected in Spain in 1967 was concentrated, and a number of chromatographic steps was used to separate a partially purified active fraction. From chromatographic mobility in TLC, gas chromatographic retention indices, and the results of various microreactions on TLC plates and in gas chromatographic subtraction loops, it was established that the active substance was an alkyl epoxide with 18 to 20 carbon atoms.

From the gas chromatographic (GC) data it was apparent that the total amount of pure attractant in the 78,000-tip extract was no more than a few micrograms, an amount insufficient for all the tests needed for adequate characterization of the chemical structure of the attractant. To obtain additional material for structural identification, the possible presence of an olefinic precursor of the attractant epoxide was explored. Treatment of a portion of the original neutral fraction with m-chloroperbenzoic acid to convert olefins to epoxides resulted in a tenfold enhancement of activity, indicating that the concentration of the olefin in the extract was considerably higher than that of the attractant itself. (This enhancement of activity by epoxidation of the hydrocarbon fraction of tip extracts was used to increase the activity of extracts for survey trapping (Bierl et al. 1971).)

The olefin in the extract was isolated and identified by microchemical reactions and combined GC-mass spectrometry as (Z)-2-methyl-7-octadecene. (Its role as a biochemical precursor of the attractant is conjectural and does not appear to have been established conclusively.) Synthesis of this olefin was

accomplished by a reverse Markownikoff addition of hydrogen bromide to 6-methyl-1-heptene, followed by a Wittig reaction with undecanal. Epoxidation of the olefin yielded cis-7,8-epoxy-2-methyloctadecane (disparlure). The natural attractant was shown to be identical with this synthetic material by gas chromatography, mass spectrometry, and bioassay. Other structurally related compounds were synthesized and tested by electroantennograms, laboratory bioassay, and field tests, but none was as active as disparlure (Bierl et al. 1970, 1972, Adler et al. 1972, Sarmiento et al. 1972, Sheads et al. 1975).

The pure trans isomer of disparlure is slightly attractive, and its effect in mixtures with disparlure appears to be a diminution of activity by dilution, with no evidence of inhibition (Beroza et al. 1971a).

The synthesis of disparlure by Bierl et al. (1970, 1972) was accomplished with an overall yield of 60 percent from 6-methyl-1-heptene, and the final product contained about 12 percent of the relatively inactive trans isomer. Several workers have modified this synthetic route or developed new ones to improve stereoselectivity; syntheses of disparlure described in the literature are discussed in a recent review (Henrick 1977).

Collins and Potts (1932) noted that removal of fatty materials from a tip extract shortened the duration of activity, possibly because the fatty substances impeded the volatilization of the attractant. However, in the early studies, addition of other materials to reduce volatilization failed to extend the period of activity (Collins and Potts 1932). With disparlure, the search for a means of regulating its volatility was more successful: The saturated triglyceride, trioctanoin, was found to be very effective as a "keeper" or evaporation suppressant (Beroza et al. 1971a). However, triolein, an unsaturated triglyceride, was ineffective in prolonging the activity of disparlure in traps. It was suggested that the loss of activity of lure in contact with unsaturates might partially account for the increase in activity and stabilization obtained upon hydrogenation of crude attractant extracts (Acree et al. 1959).

The presence of the olefin (Z)-2-methyl-7-octadecene in the tip extracts may also be a factor in the lower activity before hydrogenation. Cardé et al. (1973) have demonstrated that the presence of this compound in traps reduces the trap catch obtained when live virgin females or disparlure are used as sources of attraction; removal of this compound by hydrogenation would therefore increase the activity of the extract. Because of this inhibitory action on trap catch, it is important that any synthesis of disparlure be controlled to ensure that the content of the olefin in the final product is kept to a minimum.

At the time of the identification of disparlure as the gypsy moth attractant, the question of configuration at the chiral centers in the natural attractant was not addressed. In 1974, Iwaki et al. announced the synthesis of the enantiomers of disparlure and of its geometrical isomer, trans-7,8-epoxy-2-methyloctadecane, starting from glutamic acid. Mori et al. (1976) and Farnum et al. (1977) have also synthesized the enantiomers of disparlure, using tartaric acid and methyl p-toluenesulfinate, respectively, as the optically active starting materials. Laboratory and field tests have shown that the (+) enantiomer of disparlure, (7R,8S)-epoxy-2-methyloctadecane, is a very powerful attractant, while the (−) enantiomer does not attract male moths (Yamada et al. 1976, Vité et al. 1976, Cardé et al. 1977b, Plimmer et al. 1977a, Miller et al. 1977, Miller and Roelofs 1978). It therefore seems probable that the (+) enantiomer of disparlure is the natural sex attractant, but the optical properties of the naturally occurring compound have not yet been established.

Evolution of Formulations

Jack R. Plimmer, Barbara A. Bierl-Leonhardt, and May N. Inscoe

Introduction

The effectiveness of behavior-modifying chemicals used in pest management may depend greatly on the way in which they are formulated. Active ingredients are seldom used alone. In the simplest case, an attractant applied to a cotton wick may be an adequate bait for an insect trap. However, if a large

number of traps is to be distributed over a wide area and left unattended, a more satisfactory bait is necessary. A suitable formulation can prevent the attractant from decomposing and ensure that it retains its activity over a long period of time.

The trap catch may be affected by rate of release of the attractant from the formulation; thus the rate of release of pheromone must be optimized if the traps are to function efficiently (Flint et al. 1977). Observations in the field indicate that increasing pheromone loading beyond the optimum may result in decreased catch of male gypsy moths (Plimmer et al. 1977a).

Formulation research in our gypsy moth program has been conducted to achieve two principal goals. The first of these was to develop a formulation of disparlure for use in survey traps; the second was to develop a formulation that could be used for permeating the air with pheromone to suppress mating of the adult moths.

Adult gypsy moths emerge from late June into August in various parts of the Northeastern United States. For use in traps, a formulation must release disparlure at a constant rate over a 2–3 month period. Thus traps can be placed in the field before emergence and will require little attention during the flight season.

The air permeation technique requires maintenance of an aerial concentration of pheromone that is sufficient to disrupt the normal mating behavior of the insect. The formulation used must be effective over a 6- to 10-week period, to span the flight season with allowance for early or late emergence, and must withstand the action of midsummer temperatures and rainfall. Cost of application will be minimized if a single application of a disparlure formulation provides an adequate concentration throughout the flight period.

While research to achieve these objectives was underway, the recent synthesis of (+) disparlure provided a further challenge for formulation technology. Because the (+) enantiomer of disparlure is extremely costly, an additional criterion of success in formulating it as an attractant is economy. It is important that the amount contained in a bait formulation should be as small as possible, providing that the other criteria are satisfied.

Trapping

In the first tests with disparlure as a trap bait, solutions of lure in a volatile solvent were placed on filter paper wicks or sections of cylindrical cotton wicks (dental wicks), and the solvent was allowed to evaporate, leaving the desired amount of disparlure on the wick. However, disparlure alone at very low concentrations was not sufficiently persistent, and various materials were tested as volatility regulators. Trioctanoin was found to serve well as a "keeper." A trap with a wick baited with 0.005 μg disparlure and 5 mg trioctanoin and aged in the laboratory for 36 days caught more moths than a trap baited with a similarly aged wick treated with 10 times as much disparlure only; after 90 days there was a fivefold difference in the catch without trioctanoin compared with the catch with one-tenth as much disparlure but with trioctanoin added (Beroza et al. 1971a).

Although the addition of trioctanoin extended the life of disparlure on a wick, the rate of emission of lure was still quite variable, and a formulation that would protect the lure from environmental degradation and emit lure at a regular rate was sought. A three-layer laminated dispenser having the central lure-containing layer sandwiched between two plastic layers has proved to be very useful in this regard (Beroza et al. 1974a, 1975b). Studies on factors affecting the rate of emission of lure from these Hercon® dispensers (Bierl and DeVilbiss 1975, Bierl et al. 1976) have led to the development of dispensers having improved emission characteristics; these dispensers are widely used in survey traps. Studies on improved methods of dispensing lure for trapping are continuing.

Mating Disruption by Air Permeation

Although disparlure is relatively involatile, a solution of the material applied in a forest environment as a spray does not persist for a long period. Experiments in Russia showed that a benzene

solution of disparlure applied at a rate of 0.2 g active ingredient (AI) per hectare provided a measurable but short-term mating disruption. Biological effectiveness persisted for little longer than a week (Bednyi and Kovalev 1975).

Fortunately, during the past decade new techniques have been developed that have contributed significantly to the improvement of pheromone formulations. The growth of controlled-release technology is associated with a variety of biological disciplines. Many drugs, medicinals, fertilizers, and pesticides can be used more effectively if low-level doses can be maintained over prolonged time intervals, and many novel formulation techniques have been used to achieve this result. These include polymer coatings, starches, gelatinous coatings, rubber formulations, and many other controlled-release formulations. Many of these consist of a degradable or soluble wall or coating around the active ingredient. An alternative is the matrix formulation in which the active material, uniformly incorporated in a polymeric medium, may be leached out or diffuse into the surrounding medium or escape when the formulating medium is degraded. In all cases, the pheromone must volatilize from the controlled-release formulation at the desired rate.

In initial preseason air permeation experiments in Massachusetts, 0.64-cm squares of disparlure-treated hydrophobic filter paper were distributed by aircraft on 16-ha plots at a rate of 50 mg disparlure per hectare. This treatment suppressed trap catch almost completely for 6 days in a population of released males (Stevens and Beroza 1972), and the catch was considerably depressed 3 weeks later.

Several improved experimental formulations were tested the following year (1972) against released male insects on Dauphin Island, Ala. (Beroza et al. 1973). Captures were suppressed 99 percent for 7 weeks by a formulation of coarse cork (6/12 mesh) coated with a mixture of disparlure and Tack-Trap® adhesive and applied at a rate of 8.2 g disparlure per hectare. A molecular sieve (4A) formulation applied at the same rate began to fail about one month after application.

Preseason tests were conducted later that year in a naturally infested area on Cape Cod with released moths. Here, a fine cork formulation (11.1 g disparlure per hectare) and a microcapsule formulation (1.8 g disparlure per hectare) were effective for 6 weeks despite unusually heavy rains. Postseason tests conducted on Cape Cod showed that a molecular sieve and three microcapsule formulations suppressed catches of released males 90 percent or more when tested 6½ and 8 weeks posttreatment (Beroza et al. 1973). Thus, the concept of mating disruption was demonstrated experimentally and showed the promise of an air permeation approach. More elaborate techniques were eventually adopted.

Microencapsulation of disparlure was used as a method of prolonging its activity in many subsequent experiments. Microcapsules are tiny hollow spheres, as their name suggests, with an outer wall composed of a polymeric material. They contain a solution of the active ingredient in a suitable solvent. By varying the proportion of reactants and the amount of the active ingredient, agitation of a suitable two-phase system yields microcapsules having the desired range of particle size and concentration of active ingredient. Microcapsules thus obtained are dispersed in a liquid medium such as water, and the suspended capsules can be sprayed through normal pesticide application equipment provided that the nozzle size is compatible with the diameters of the microcapsules. This simple formulation can be improved by the addition of thickeners that are inert ingredients and that will maintain a uniform dispersion of microcapsules throughout the medium and prevent settling in the tank of the spray application equipment. Surface active agents that promote wetting and improve the performance of the formulation are also useful additives. A sticker must be added to attach the capsules to the foliage of a crop or forest canopy. Suitable stickers and other additives must not alter the performance of the microcapsules—that is, the rate of release of the pheromone from the formulation should not be substantially affected by the additives. The rate of release of pheromone is a most important parameter that governs the performance of the formulation.

To establish dose/response relationships, broadcast applications of microencapsulated disparlure at rates

of 5.0 to 15.0 g per hectare were applied in 1973 to 16-ha test plots in central Pennsylvania using insects placed in the plots as pupae (Cameron et al. 1974, Schwalbe et al. 1974). The formulation used was one of those tested in 1972 (Beroza et al. 1973) and was a slurry consisting of: 17.6 percent gelatin-based microcapsules (about 80 percent of which were 100–250 μm in diameter) containing a 2.2 percent solution of disparlure in xylene, 2 percent of UCAR Latex 680® as a sticker, 29 percent of aqueous 1 percent hydroxyethylcellulose, 1.7 percent of 1 percent aqueous potassium hydroxide, and 49.7 percent water. The microcapsules were prepared as a 27 percent aqueous slurry by the National Cash Register Company (NCR). The results during the season suggested that disparlure formulations would reduce mating for 6 weeks after application. The highest rate, 15.0 g per hectare, appeared to suppress mating sufficiently to prevent an increase in population (Cameron et al. 1974, Schwalbe et al. 1974).

In a large-scale field trial (60 km²) in a natural infestation in Massachusetts the same year (Beroza et al. 1974b), substantial mating disruption was observed up to 5 weeks after application of the same formulation at 5 g per hectare. In addition, there was a significant reduction in egg-mass counts. These experiments were valuable in defining the amounts of disparlure necessary to maintain mating disruption at an adequate level.

Through 1974 and 1975, large field tests had been restricted to two microencapsulated formulations of disparlure, one with a gelatin-based wall and the other with a nylon-based wall. In 1976, on the basis of laboratory results, several new formulations were included in the field program, and these were tested in Maryland and Massachusetts (Plimmer et al. 1977b). The formulations selected are shown in table 6.4–1. The NCR capsules were plastic-coated, gelatin-walled capsules encasing a 3:1 xylene-amyl acetate solution of 2.2 percent or 11 percent disparlure. Because the wall material represents 10 percent of the capsular weight, the disparlure content of the capsules was 2 percent and 10 percent, respectively. Capsules were suspended in water containing a thickening agent and an adhesive or sticker to hold the capsules onto the

foliage. The sticker for the 1975 NCR formulation was 1 percent Rhoplex B–15® and that for the 1976 NCR formulations was 1 percent RA 1645® plus a surfactant, 0.1 percent Triton-X 202®. The NCR material designated as 4 percent in table 6.4–1 was a 75–25 mixture of the 2 percent and 10 percent materials.

The matrix formulation, designated MGK, was an aqueous slurry of particles of a paraffin wax/inorganic salt matrix containing 2 percent disparlure together with a thickener and adhesive. The Conrel fibers were hollow 8 mil i.d. plastic fibers, 2.3 cm in length and filled with a solution of 30 percent disparlure in hexane. All formulations, with the exception of the Conrel hollow fiber (for which special equipment had to be used), could be applied by conventional spray application equipment and were applied from spraying system 8010 tips on spray boom nozzles.

For field testing, the formulations in table 6.4–1 were applied to 16-ha test plots at a rate of 20 g of lure per hectare (Plimmer et al. 1977b). Each formulation was applied to four replicate plots in both Massachusetts and Maryland; eight control plots were established in each State. Mating in treated plots was compared with that in the control plots. Virgin female moths were placed in the plots and retrieved after 3 days. Recovered insects were dissected to determine whether sperm was present, and any egg masses deposited were collected and examined for the presence of fertile eggs. The results of the 1976 field tests are given in table 6.4–2. The seven formulations tested showed the same ranking in both States, although the differences among the top performers were not statistically significant.

The 1976 2 percent NCR formulation reduced mating 96 percent in Maryland and 83 percent in Massachusetts. Corresponding figures for the next best performers, the 1976 4 percent NCR formulation, were 91 percent and 76 percent, respectively. It has been postulated that the mating disruption technique will depend on the density of the native moth population (Beroza and Knipling 1972) and that it will become more efficient in sparse populations, where the chance of random mating is least. Therefore, it seems likely that the difference between

Table 6.4-1.—*Formulations used in field testing, 1976*

Formulation designation	Manufacturer	Percent lure in particles	Particle size (μ)
1976-NCR-2	National Cash Register	2	50-250
1976-NCR-4	National Cash Register	¾ of 2 + ¼ of 10	50-250
1976-NCR-10	National Cash Register	10	50-250
1975-NCR-2	National Cash Register	2	50-400
Stauffer	Stauffer Chemical Co.	¾ of 2 + ¼ of 10	10-40
MGK	McLaughlin Gormley King Co.	2	100-200
Conrel	Albany International Co.	30	Fibers

Source: Plimmer et al. 1977*b*.

Table 6.4-2.—*Mating reduction in 1976 field tests*

Material[1]	Maryland tests				Massachusetts tests			
	Number of moths dissected	Number of moths mated	Percent mated	Percent reduction in mating	Number of moths dissected	Number of moths mated	Percent mated	Percent reduction in mating
Controls	260	124	47.7	—	644	315	47.9	—
1976-NCR-2%	101	2	2.0	96	364	29	8.0	83
1976-NCR-4%	121	5	4.1	91	273	31	11.4	76
1976-NCR-10%	149	7	4.7	90	270	37	13.7	71
1975-NCR-2%	91	9	9.9	79	292	45	15.4	68
Conrel	76	7	9.2	81	282	59	20.9	56
Stauffer	128	31	24.2	51	356	105	29.5	38
MGK	132	49	37.1	22	358	129	36.0	25

[1]Eight control plots; all formulations four plots each.
Source: Plimmer et al. 1977*b*.

the performance of the formulations at the two locations was a reflection of the difference in populations.

Tests conducted in 1975 using the 2 percent NCR microcapsules on a large scale in Maryland and in Massachusetts had given results that were parallel to those in the 1976 tests. In Maryland, mating reduction was 80 percent, and in Massachusetts mating was reduced by 68 percent. Thus the 1975 and 1976 results with the 1975 formulation were comparable, and it was felt that the new sticker, RA 1645, and a decrease in capsule size had improved the performance of the 1976 2 percent NCR formulation sufficiently to account for the differences in results.

Laboratory Testing

Although the ultimate test of a formulation is its performance in the field, the number of formulations that can be included in a field test is severely limited. For this reason, preliminary laboratory studies on emission characteristics, effects of formulation adjuvants, and the role of environmental factors are needed to organize the many parameters that can be varied during the preparation of a controlled-release formulation and to guide in the selection of suitable formulations and application rates to be used in the field (Bierl and DeVilbiss 1975, Bierl et al. 1976).

Before selecting the formulations for the 1976 field tests, for example, the effects of a number of variables

on the performance of 20–30 candidate formulations were studied (Plimmer et al. 1977*b*). In assessing the longevity of a formulation, samples on microscope slides were aged in the laboratory or outdoors. Both rate of pheromone release and amount of pheromone remaining in a formulation were determined as a function of time of aging. Emission rate provided a better guide to performance than did measurement of residual pheromones; with some microcapsular formulations, emission of disparlure ceased although substantial amounts of pheromone still remained in the capsules. Ideally, the release rate of disparlure from microcapsules should undergo approximate exponential decay as the concentraton of disparlure decreases. In practice, it was found (Plimmer et al. 1977*b*) that there was occasionally a burst of pheromone immediately after application, and that after this initial burst, the slope of the plot of release rate vs. time decreased. From microphotographs it also appeared that some gelatin microcapsules lost their contents rapidly and became distorted, possibly as a result of high humidity.

In the field, the effective lifetime of a formulation is drastically reduced by weathering; in some field trials (without sticker), microcapsules exposed to sun, wind, and rain lost half their lure in 19–34 days, compared with 123 days under laboratory conditions.

A sticker in a formulation forms a coherent adhesive film after application. This film aids in reducing loss of pheromone by weathering, but it may also affect the rate of emission of pheromone and thus alter the peformance of a formulation. It is therefore necessary to measure pheromone release rates after the addition of sticker to the formulation. The presence of 2 percent of sticker RA 1645 in the 1976 NCR and MGK formulations reduced the emission rate of disparlure by over 50 percent in laboratory tests. To obtain more efficient pheromone release, the concentration of this sticker used for aerial application was reduced to 1 percent.

To test the predicted behavior of a formulation under environmental conditions, the rate at which pheromone was emitted from a microencapsulated formulation that had been applied to a grass-covered plot was measured (Caro et al. 1977). Air sampling equipment was used to trap disparlure at several heights above the plot, and wind speed, temperature, and relative humidity were measured. Thus the effect of these environmental variables on the concentration of disparlure in air could be evaluated. Similar experiments were undertaken to investigate the concentration of disparlure within the forest canopy after a microencapsulated formulation had been applied by air. There was a considerable difference between disparlure concentrations at 0.5 m and 15 m above the ground, with a greater concentration of pheromone near the ground than in the higher strata (Plimmer et al. 1978).

Current Status of Formulation Research

The three-layer laminated-plastic dispenser has proved to be adequate as a source of disparlure for survey traps. It emits the pheromone at a constant rate over a long period, can be easily handled, and is quite durable. Because (+) disparlure will probably replace racemic disparlure as the trap bait, it is necessary to devise formulations that will utilize little more than 1 mg of attractant, compared with 6 mg used in the standard dispenser that is manufactured for the USDA survey program. New plastic-laminated dispensers are now being tested. Measurements of emission rates and loss of lure content on aging will indicate the optimum design parameters for a modified formulation.

Microencapsulated formulations have been evaluated for mating suppression. The NCR 4 percent and 2 percent formulations applied at 20 g AI per hectare appear promising for potential areawide aerial application to suppress mating throughout the flight season in areas that have low-level infestations. A modification of the 2 percent NCR 1976 microcapsule with improved emission characteristics will be field tested in the 1978 season.

Several controlled-release formulations based on laminated plastic dispensers, hollow fibers, etc., are under study but have not yet been definitively evaluated as potential mating suppressants. Effective mating disruption was obtained when a grid of laminated dispensers was placed in the forest (see

Webb et al., Disruption Along the "Leading Edge" of the Infestation, this section). This technique may be valuable for suppression of mating in areas where spray application is undesirable.

Although sources of pheromones, such as microcapsules and dispensers, may release lure uniformly over a long period, little information exists concerning natural sinks which may rapidly absorb or take up disparlure from the air. Possibly, the surfaces of soil, leaves, and plants may adsorb the material. These factors will affect the efficient use of pheromones, and their role must be elucidated before formulations that will be effective in air permeation techniques can be developed fully.

The Use of Attractants in Traps

Disparlure-Baited Traps for Survey and Detection
Charles P. Schwalbe

Pheromone-baited traps have demonstrated their usefulness in programs designed to detect and delimit populations of gypsy moth and have potential value for monitoring populations and estimating density. Necessarily, trap designs and deployment patterns will vary according to the objectives of the trapping program. For example, efficient traps (which capture a large proportion of male moths orienting to them) are desirable for surveys demanding a high probability of detection. Traps placed for detection survey in areas not currently known to be infested should have a high efficiency. In this case, trap capacity need not be large, because most infestations are likely to be rather sparse when detected. The intent of the survey is to determine qualitatively the presence of infestation; quantitative information may be less important at this stage. On the other hand, traps used for monitoring established infestations must have a large capacity to reflect changes effectively (perhaps over several orders of magnitude) in population density. A high degree of trap efficiency may not be necessary for such applications. If efficiency is defined (and constant), insect density can be determined from trapping data (Granett 1974).

Traps baited with female moths or a sex attractant have been used routinely in support of a variety of containment, control, and regulatory programs. Collins and Potts (1932) tested a number of traps and various types of extracts prepared from excised abdomens of female moths. This exploratory work established the base of technology upon which future trapping efforts would grow. "Assembling cages" (traps) were used systematically as early as 1932 (Burgess 1944). They consisted of an inverted cone of oiled cardboard into which was inserted cotton batting or rolled, corrugated cardboard impregnated with a benzene or xylene extract of female abdominal tips. The cone was nailed to the center of a 30×30 cm square of Tanglefoot® applied to a tree. Moths captured in such traps were considered evidence of nearby infestation, and intensive visual scouting for egg masses was focused in these areas.

These early survey programs utilized systematic trap placement methods; infested sections of Pennsylvania and New York were trapped on a grid pattern with a trap density of about one trap per 166 ha (Corliss 1948). However, a larger part of these trapping programs consisted of placing traps at 0.8-1.6 km intervals along traveled roads (Corliss 1948). Approximately 70,000 traps were deployed in this manner from 1944-48 in Massachusetts, Connecticut, New York, Pennsylvania, and New Jersey as part of an effort to contain gypsy moth populations in the generally infested New England States (Corliss 1948). Egg-mass surveys, coupled with male moth trapping surveys, were utilized to map the distribution of the insect and to identify treatment sites within the suppressive area of the barrier program. Until the termination of the containment program in the 1950's traps were used almost exclusively in the Northeastern United States to detect infestations forward of the barrier zone.

As long-distance travel and shipping of materials and commodities increased, the likelihood of establishment of new infestations far removed from the Northeastern United States also increased. Early detection of new infestations was necessary if the insects were to be kept from building to damaging levels. Therefore, inexpensive, effective, and uniform

traps and lures that could be used in large numbers over wide geographic areas were necessary. A variety of technical problems required solution to develop this capability, and the operational trapping system was frequently modified to implement improvements in technology. Many changes in trap design were brought about by the unavailability of construction materials and bulkiness of the early traps. Other design modifications were probably introduced to increase trap efficiency.

Castor oil resin, Tanglefoot®, and Tack-Trap® are sticky adhesives that have been employed to ensnare moths that enter the traps. Certain formulations had a tendency to form a film and lose tackiness and required periodic "combing" to retain effectiveness.

The type of bait used and the methods for dispensing it from the trap were continually under scrutiny. Extracts of female abdominal tips were widely used. This created obvious logistical difficulties, and much effort and expense went into obtaining adequate quantities of extract to support survey programs. Inadequate quality control of these preparations often resulted in the field use of inactive batches of attractant. Chemical methods for enhancing the potency of the bait were evaluated (Collins and Potts 1932). Consequently, effort was intensified to identify the natural sex attractant, and the results are detailed by Inscoe and Plimmer (Chemistry of Isolation, Identification, and Synthesis, this chapter). Improvements in formulation to prolong activity of the bait in the trap are described by Plimmer et al. (Evolution of Formulations, this chapter).

A tabulation of the various trap designs and attractant formulations that have been used in gypsy moth survey programs since 1896 is presented in table 6.4-3.

Annually since 1972, systematic survey for gypsy moth has been conducted over most of the United States where susceptible forest types occur. In 1972, trap densities varied from 1 trap per 2.6 km^2 to one trap per 117 km^2; trap density was adjusted according to distance from the generally infested area of the United States and pest risk. Since artificial gypsy moth movement is normally associated with movement of man or his commodities, trapping effort is focused on areas that pose a high pest risk, such as campgrounds, recreational sites, mobile home parks, logging mills, and residential areas. Now most detection survey is conducted on a grid pattern of one trap per 7.8 km^2. If resources are inadequate to survey a given area at this density, the area is subdivided into sections that are trapped on a rotation basis; traps are only placed in those localities in which stands of susceptible forest type are found. In this fashion, a given susceptible part of each area is trapped at least every 3 to 4 years. These programs have been responsible for determining the presence of male gypsy moths in the field. From 1972 to 1977, 113 new county records for gypsy moth have been established (see table 6.4-4).

It is generally assumed that many of the male moths captured in detection survey traps are "hitchhikers" associated with travel by vacationers and movement of recreational vehicles and do not represent evidence of an established infestation. Locations where moths are detected are resurveyed the following year with a more dense trap array (to be described later) to determine if a population is established and, if so, to delimit its range. Using the presence of egg masses (or other life stages) and/or male moths recovered in traps at a given location for 2 consecutive years as the criterion for infestation, nine infestations in North Carolina, Florida, Ohio, Wisconsin, California, Michigan, Illinois, and Virginia have been discovered and delimited since 1972.

While the repeated capture of male moths or the observation of immature stages gives good evidence for the existence of a gypsy moth population at a given location, the delimitation of the boundaries of the infestation requires intensified effort. Visual scouting for egg masses is of little value at sparse population levels because of the very low probability of finding a significant proportion of egg masses. Similarly, the likelihood of observing other immature life stages of the insect at such densities is remote. Highly attractive pheromone-baited traps deployed in a grid arrangement over the areas suspected to be infested permit identifying the focus of infestation. The probability of capturing a male moth in a trap is related, in part, to

Table 6.4-3.—*Summary of pheromone-based trapping methods used in gypsy moth survey, 1896–1977*

Period used	Trap design	Bait/dispenser	Adhesive	Reference
1896–1917	Shaw trap. 30.5 cm² baseboard with two intersecting 18×30.5 cm boards notched together at center forming 4 vertical wings	Live virgin female in screen box	Castor oil resin on inside surface of box	Forbush and Fernald 1896
1913–28		Live virgin female moths held in screened box	Castor oil resin or Tanglefoot® painted around box on tree trunk	Collins and Potts 1932
1928–30		Cotton wad containing extract of 15–30 virgin female abdominal tips; placed in inverted metal cup	Tanglefoot® painted on tree trunk around bait dispenser	Collins and Potts 1932
1930–44		Cotton wad containing extract of virgin female abdominal tips; placed in inverted oiled cardboard cone	Tanglefoot® painted on waxed cardboard and nailed to tree	Holbrook et al. 1960
1936–48	Pott's trap (30.5×15.3 cm stove pipe)	Abdominal tip extracts, hydrogenated tip extracts or gyptol applied to cotton wick inside trap	None or Tanglefoot® on a cardboard liner	Holbrook et al. 1960
1945–48	Graham trap (18×36 cm fruit juice can) with inverted screen cone ends	Hydrogenated abdominal tip extract applied to roll of corrugated paper (2.5×7 cm)	Tanglefoot® on a cardboard liner	Burgess 1950
1949–54	Graham trap with inverted cardboard cone ends	Hydrogenated abdominal tip extract applied to roll of corrugated paper (2.5×7 cm)	Tanglefoot® on a cardboard liner	Holbrook et al. 1960
1955–60	Graham trap with inverted cardboard cone ends	Hydrogenated abdominal tip extract applied to roll of crepe filter paper (1.25×7 cm)	Tanglefoot® liner	Holbrook et al. 1960
1961–63	Johnson trap—227-ml paper hot drink cup with 11-mm half-moon entrance port on small end and 3.2-cm circular hole in cover	Hydrogenated abdominal tip extract applied to 10×0.6 cm cotton wick; gyplure	Tanglefoot® smeared on inside of trap	
1962–68	Johnson trap—227-ml paper hot drink cup with 11-mm half-moon entrance port on small end and 3.2-cm circular hole in cover	25 μg gyplure or extract of 12 abdominal tips on cotton wick	Tanglefoot® smeared on inside of trap	Jacobson et al. 1960
1969–70	Johnson trap—227-ml paper hot drink cup with 11-mm half-moon entrance port on small end and 3.2-cm circular hole in cover	Abdominal tip extract applied to cotton wick	Tack-Trap®	
1971–72	Johnson trap—227-ml paper hot drink cup with 11-mm half-moon entrance port on small end and 3.2-cm circular hole in cover	20 μg disparlure applied to cotton wick	Tack-Trap®	Bierl et al. 1970
1973	Johnson trap—227-ml paper hot drink cup with 11-mm half-moon entrance port on small end and 3.2-cm circular hole in cover	100 μg disparlure with 2 mg trioctanoin keeper	Tack-Trap®	Beroza et al. 1971*h*
1974	Johnson trap—227-ml paper hot drink cup with 11-mm half-moon entrance port on small end and 3.2-cm circular hole in cover	300 μg disparlure with 2 mg trioctanoin keeper or 2.5×2.5 cm Hercon® dispenser with 2 percent disparlure	Tack-Trap®	
1975–77	Delta trap—18.5×10.2 cm triangular-shaped paperboard, polyethylene coated	2.5×2.5 cm Hercon® dispenser with 5 percent disparlure	Tack-Trap® applied to two of three inside trap walls	Beroza et al. 1975*h*
1977	Delta trap—18.5×10.2 cm triangular-shaped paperboard, polyethylene coated	Cotton wick containing 100 μg (+) enantiomer of disparlure was used on limited basis to survey small isolated infestations	Tack-Trap® applied to two of three inside trap walls	

Table 6.4-4.—*Summary of gypsy moth detection survey with pheromone-baited traps, 1972-77*

Year	Number of traps placed	Number of States trapped	Number of new county records outside quarantine zone	Number of States outside quarantine zone in which moths were captured	Reference
1972	120,000	31	59	11	USDA 1972
1973	65,000	44	67	15	USDA 1973
1974	70,000	31	14	10	USDA 1975
1975	73,000	31	16	9	USDA 1976
1976	84,000	35	12	10	USDA 1977
1977	95,000	40	11	7	USDA 1978

the distance the insect is from the trap. Also, it is known that the number of insects trapped in a given array of traps is proportional to the original pupal population density (Granett 1974). Those traps in a grid pattern with the highest number of captured insects represent the center of the infestation. Presumably, insect population density will decline outward from the center of the infestation and, consequently, recoveries of moths will also decline. Therefore, in a stylized system, isometric lines connecting traps that captured similar numbers of male moths should isolate the focal point of the infestation and illustrate the declining gradations of population density outward toward the periphery of the infestation. If the effectiveness of a given array of traps is known, and the population is reasonably confined to an isolated area, the extent of the infestation can be determined from the pattern of moths captured in traps. On the basis of the studies discussed below, this method of delimitation will be pilot tested by USDA and cooperating State agencies in infestations in Wisconsin, Washington, Virginia, and Michigan in 1978.

Delimitation surveys are conducted by placing a grid of traps throughout the area in which infestation is suspected so that appropriate control measures can be accurately applied if necessary. For the past several years, trap density in these surveys has been 16 traps per 2.6 km^2 in 23.4 km^2 centered over the area where the insects were captured the previous year. The 41.6 km^2 bordering the central 23.4 km^2 are trapped at the rate of 9 traps per 2.6 km^2. Any 2.6 km^2 unit in which

male moths are trapped is generally assumed to be infested. Most operational experiences have been with the delimitation of rather small, isolated infestations, and in those cases, only a few traps in the array captured moths.

With the exception of approximately 300 traps baited with the (+) enantiomer of disparlure in 1977, traps have been baited with racemic disparlure in all survey programs since 1971. The recent discovery that the (+) enantiomer of disparlure is about 10 times more attractive than the racemic mixture (Plimmer et al. 1977a, Cardé 1977) stimulated a series of experiments designed to provide information with which to phase the improved trap bait into future male moth detection and delimitation survey programs. Because the intertrap distance (ITD) and pheromone release rate of traps on a grid pattern are likely to affect trap effectiveness, these parameters varied. Additionally, traps baited with 2,000 μg of the (±) disparlure were compared with those baited with the (+) enantiomer. Pheromone was applied to cotton wicks suspended in the center of delta traps. Field-collected male moths (1 day old) were released at the center of the trap grids, and the number of insects recovered at the various trap locations was recorded. Recovery in traps of moths released in the plots is given in table 6.4-5.

Recovery of released insects decreases as ITD increases. Therefore, the probability of capturing male moths is a function of trap density. Furthermore, it appears that increasing the attractant concentration cannot compensate for the reduced

Table 6.4–5.—*Percent recovery of male moths released into trap arrays with different intertrap distances (ITD) and attractant concentration, Fall River, Mass., 1977.*

ITD (m)	Traps per plot	Attractant (μg)			
		10(+)	100(+)	1000(+)	2000(±)
88	64	21.5	35.4	27.0	9.4
175	36	8.6	7.9	16.7	1.1
350	16	2.8	5.1	0.6	0.9

Figure 6.4–1.—*Percent capture of male gypsy moths in traps at various distances from the release point. All traps were baited with 100 μg (+) disparlure.*

capture of moths in grids of traps widely spaced. In fact, traps baited with 1,000 μg (+) disparlure generally captured fewer insects than those baited with 100 μg (+) disparlure. The optimum concentration of 100 μg (+) disparlure has been verified in other tests which also demonstrated that changing the concentration of (±) disparlure in traps has no appreciable effect on attractancy (Plimmer et al. 1977*a*). It is not known why catch in traps baited with more than 100 μg (+) disparlure decreased. In nearly every plot more insects were captured in traps baited with (+) disparlure than those in which the attractant was (±) disparlure, clearly showing the greater trapping effectiveness that can be obtained by using the (+) form of disparlure. The percent recoveries reported in table 6.4–5 are based on male moths that were liberated from a central release point and at the maximum possible distance from the traps on the grid. If the insects had been released randomly throughout the grid, the recoveries would likely have been higher because many would have been released closer to individual traps.

It is illustrative to examine the pattern of male moth capture in the various arrays. In figure 6.4–1, the frequency of moth capture at a given trap location is plotted as a function of the distance of the trap from the release point. The data are taken from the three plots (ITD=88, 175, and 350 m) in which traps were baited with 100 μg (+) disparlure (see table 6.4–5). In all cases, the largest numbers of captured moths were found in traps closest to the point of release. As the distance from the release point increased, percent capture decreased. In plots with an ITD of 88 m on an 8×8 grid, approximately 23 percent of the released

population (from a total of 35.5 percent recaptured) was captured by the closest traps, which were 62 m from the release point. Four percent were recovered in traps 140 m from the center of the plot, and catches gradually declined from about 1 to 0.2 percent at trap locations 183 m to 427 m from the release point, respectively. A similar pattern of capture was recorded in plots where the ITD was 175 m in a 6×6 grid. Here, approximately 5 percent of the released males were caught 115 m from the release site. Less than 1 percent were captured 255 m from the center of the plot. Very few males were captured in traps farther than 255 m from the release location, and none was found in traps located 575 m from the central release site. The traps in plots with an ITD of 350 m in a 4×4 grid captured few of the released moths. The closest traps (200 m) recovered about 3.5 percent, while those at 450 m and 600 m caught only 0.62 percent and 0.38 percent of the released population, respectively.

In comparing the curves from the 88 and 350 m ITD plots (figure 6.4–1), it appears that as trap density decreases, the probability of capturing moths farther

from the release point increases. Evidently male moths do not disperse as far in the presence of disparlure-baited traps before they are captured. Also, it is likely that more males were captured in traps far from the release point in the 350 m ITD plots than in the 88 m ITD plots because the dispersing population was greater; in plots with 88 m ITD, more males were removed from the population by the central traps before dispersal to the more distant trap locations. However, in either case, less than 1.0 percent of the released population was captured further than 450 m from the plot center.

A similar experiment was conducted over larger areas with greater trap spacing to simulate intensified detection survey that may be used around an area that has a history of moth finds or is in a high risk category (campground, mobile home park, etc.). A 65 km^2 forested area was overlaid with a grid of transect lines 805 m apart, creating a 10×10 grid of trap sites. Two delta traps, separated by 15–20 m and baited with 100 μg (+) or 200 μg (±) disparlure, were placed near each grid point. Male moths (laboratory reared) were released near the centers of four separate sections (2.6 km^2 areas). One smaller plot (2.3 km^2) was also established in which the ITD was 304 m in a 6×6 grid. Here also, paired traps separated by 20 m were placed at each point, and male moths were released in the center of the plot. Results are presented in table 6.4–6. In these tests, capture of released males in the 805 m ITD plot was 0.92 percent and 0.67 percent in the (+) and (±) disparlure-baited traps, respectively. With the closer trap spacing, 3.3 times more moths were captured in traps baited with the (+) enantiomer than in those containing (±) disparlure (12.7 percent vs. 3.8

percent) and the recapture rates were 13.8 and 5.7 times those recorded with an ITD of 805 m. From the data, it appears that the superiority of the (+) enantiomer over (±) disparlure is not as great as observed in test plots with closer trap spacings.

The distribution of captured insects in the plots with ITD=805 m is shown in figure 6.4–2. The distributions of recoveries surrounding the four release sites have been overlaid on a single 6×6 trap grid for clarity. The patterns of capture of moths in the (+) and (±) disparlure-baited traps was very similar. Approximately 78 percent of the captured moths were recovered within 570 m of the release point; only 22 percent of those captured moved out of the section in which they were released. No insects were captured in traps "two sections" away from the release points (not shown in figure 6.4–2).

In summary, approximately 1 percent of the released moths were captured in a grid of traps separated by 805 m. Most insects were recovered from those traps closest to the release point and the

Table 6.4–6.—*Capture of released laboratory-reared male moths in traps baited with 100 μg (+) or 200 μg (±) disparlure and placed in grids with ITD=805 m and 305 m, Mount Pleasant, Mich., 1977*

	Percent captured	
Attractant	805 m	305 m
100 μg (+)	0.92 (n=21)	12.7 (n=152)
200 μg (±)	.67 (n=18)	3.8 (n=45)
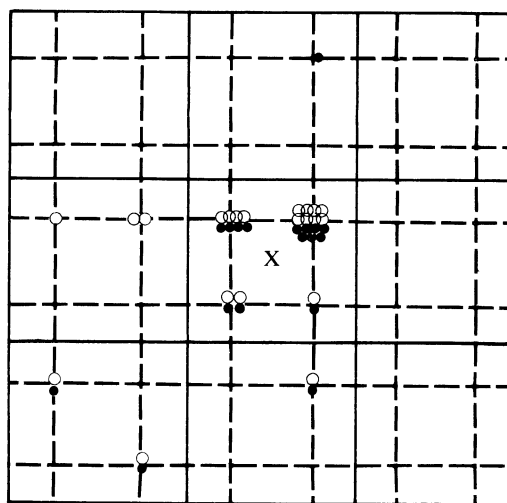
Figure 6.4–2.—*Distribution of male gypsy moths in a 6×6 array of traps with ITD=1.3 km. Open circles indicate individual moths captured in (+) disparlure-baited traps and closed circles show locations of males in (±) disparlure-baited traps. The dotted line shows the trap grid and X indicates the release point. (Mount Pleasant, Mich., 1977.)*

probability of capturing moths more than 1,200 m away is slight.

A variety of factors, such as predation, wind speed and direction, temperature, precipitation, geographical and topographical features, male moth population quality, sex ratios and age classes, influences male moth dispersal activity, responsiveness to pheromone sources, and survival. These in turn affect the probability of capture in traps. The significance of each factor on moth-trap interaction must be quantified and integrated into a comprehensive model to ensure that interpretation of trapping data is reliable and accurate. The effectiveness of trap arrays in detecting and delimiting populations of gypsy moths will vary according to the relative influence of those factors.

Disparlure-Baited Traps for Population Reduction or Eradication
E. Alan Cameron

Knipling and McGuire (1966) developed a series of models to test the theoretical effects of the use of sex attractants for insect control. One of these models (model II) assumed that "... all males attracted ... will be killed immediately by being trapped ...." Using the principles developed in the 1966 paper, Beroza and Knipling (1972) presented a model specifically for the gypsy moth, in which traps baited with disparlure were to be used. In the early 1970's, several studies were undertaken to test this model (E. A. Cameron 1971, 1973, Beroza et al. 1973).

During summer 1970, eight male moths were captured in Somerset County in southwestern Pennsylvania in two survey traps separated by approximately 3 km. Subsequent searching by various individuals revealed the presence of 10–15 new egg masses, and later at least one egg mass that had been deposited in 1969, within an area of about 6 ha around one of the traps. No egg masses were found in the vicinity of the second trap catch or elsewhere in the general area. Since this area was about 125 km away from the closest known infestation and was presumably a new, small infestation, with a sparse population of insects, it was chosen as a site from which to attempt to eradicate the gypsy moth.

Because this infestation was outside the then-quarantined region of the State, 73 ha centered on the area known to contain egg masses were sprayed in early June 1971, with Sevin 4-Oil®. Yellow cardboard tube traps (7.5 cm long and 2.5 cm in diameter, glassine-lined and coated internally with Tack-Trap®, and containing cotton wicks baited with 10 μg disparlure) were dropped 3 weeks later from the air over about 22 km^2 at a rate of 610 traps per square kilometer. Subsequent monitoring revealed that the infestation was more generally distributed than had been previously suspected, that fertile egg masses had been deposited within the treated area, that the area previously known to have egg masses present was, indeed, a "hot spot," and that the pesticide treatment did not eliminate the resident population (Cameron 1971).

In 1972, the area over which traps were dropped was enlarged to about 35 km^2 and the trap density increased to 2,800 per square kilometer. Three 40-ha areas within the drop zone and known to contain fertile egg masses received traps at the rate of 8,400 per square kilometer (E. A. Cameron 1973). These traps had 500 μg disparlure per trap incorporated directly into the Tack-Trap®. Numerous males were captured in traps, and postseason egg-mass counts ranged as high as 500 per hectare in one small area (which was within the trap drop zone in both years).

Other tests were conducted in 1971 and 1972 that utilized hand-placed or aerially dropped baited traps in areas where natural populations were below detectable levels. Populations were simulated by introduction of pupae or adults into the test areas and monitoring the results. Results were erratic and not encouraging. In only one test was mating significantly reduced in treated areas; in other tests no significant differences were measured, and in some cases mating was actually increased in treated plots (E. A. Cameron 1973).

While there was some indication that lure emission from the Tack-Trap® carrier in 1972 was less than desired, it was clear that the traps then available and the airdrop methods used to distribute them over areas with either sparse natural populations or simulated populations were not capable of reducing

mating success enough so that a population reduction would result in the next generation. In fact, the incipient natural infestation grew. For these reasons in part, further tests with baited traps for population reduction or eradication were abandoned by the team of researchers at the Pennsylvania State University.

Beroza and co-workers carried out a preseason test on Dauphin Island, Ala., during April–June 1972. Male moths, reared at the Gypsy Moth Methods Development Laboratory at Otis Air Force Base, Mass., and shipped south as pupae, were released in a plot into which tube traps (containing 500 μg disparlure incorporated into the Tack-Trap® coating) had been aerially dropped at 25 per hectare. Recapture of males released in the plot was reduced by 94 percent when compared with recapture in an untreated plot, an encouraging result even though there was concern over the lure emission rate and consequent effectiveness of the tube traps (Beroza et al. 1973). The authors also recognized the artificiality of using laboratory-reared insects, a distorted sex ratio, and an artificial dispersion of test insects.

Gypsy moth detection surveys conducted annually in the area of Dayton, Va., during 1972–74 produced positive results (table 6.4–7), even though intensive visual searches for egg masses were consistently negative. Repeated recovery of adult insects in the area was taken as evidence that an incipient infestation existed. In 1975, the U.S. Department of Agriculture and the Virginia Department of Agriculture and Commerce cooperated in an intensive program to eradicate the infestation through

the use of mass trapping. A total of 11,281 delta traps, baited with disparlure in Hercon® dispensers, were hand-placed in June over about 39 km². Trap density was about 25 traps per hectare in 5.2 km² in and around the town of Dayton. The 31–34 km² peripheral area was trapped at the rate of 7–8 traps per hectare. Similar trap arrays were planned for subsequent years until no male moths were captured. Only one male gypsy moth was captured in the traps that season, so a greatly reduced number of traps have been placed since then. No more male moths have been captured, and the area is currently considered to be uninfested, although it is not really possible to claim eradication as a direct result of the trapping program (Schwalbe 1978).

There is little current interest in the use of diparlure-baited traps for gypsy moth population reduction or eradication, for several important reasons. Some of the traps used are not very efficient (Mastro et al. 1977), and designs identified as among the more efficient are not amenable to aerial distribution. There is still much to learn of male behavior (see Precopulatory Sexual Behavior of the Adult Gypsy Moth, this chapter), and in particular, if or how that behavior may be modified by a trap. Further, many of the assumptions on which the model of Beroza and Knipling (1972) was based are inaccurate. No one has presented an improved model that would encourage pursuit of this method of gypsy moth control. Until such a model is developed, it is not likely that mass trapping of gypsy moth males for purposes of control or eradication will receive much more attention.

Table 6.4–7.—*Results of trapping programs conducted near Dayton, Va.*

Year	Number of traps placed	Number of males captured
1971	3	0
1972	10	4
1973	20	12
1974	20	11
1975	11,281	1
1976	20	0
1977	20	0

Source: Schwalbe 1978.

Evaluation of Disparlure-Baited Traps

Victor C. Mastro

Trap Efficiency

Gypsy moth traps have undergone a continuing metamorphosis over the past 50 years (Disparlure-Baited Traps for Survey and Detection, this chapter). To improve effectiveness, lower operational cost, and ease the burden of the field worker, changes in trap design continue. Recently, identification of the (+)

enantiomer of disparlure as a more powerful attractant than the racemic mixture has increased the effectiveness of traps used in survey and detection programs. In part, at least, this is because of the demonstrated increase in trap efficiency (Cardé et al. 1977b, c, Plimmer et al. 1977a).

The term "trap efficiency" is used here to describe a trap's success in capturing male moths that have oriented to it. Many factors may influence trap efficiency, including physical characteristics of the trap, trap placement, bait, and rate of release of the bait from a dispenser. In addition, the interaction of these factors with male sexual behavior and environmental influences that affect male behavior or the trap directly also may influence the performance of a trap.

Physical characteristics of a trap (shape, size, color, and the location of entry ports) will affect its performance. However, the sexual behavior of males (reviewed by Doane 1976 and Richerson 1977) must also be taken into consideration when designing a trap for maximum efficiency. Male gypsy moths, when orienting to a pheromone source, seldom fly directly to it. As part of a sequence of behavioral steps, males land on a substrate near the source and begin a rapid wing-fanning, walking behavior. Generally, they continue this behavior until the point of pheromone emission is located or the search is terminated. Males terminating search fly rapidly away and rarely reinitiate a search at that site. The period of close-range searching behavior is very short and, although exact figures are not available, probably averages less than a minute.

In previous studies (Mastro et al. 1977) it was found that standard survey traps (baited with racemic disparlure) were not as efficient in "capturing" male moths as were virgin feral females. These comparisons were made by using observations of male behavior when attracted to traps (females). This technique identified which traps were the most efficient and also identified how traps could be designed or modified to take advantage of male searching behavior and to increase trap efficiency.

Poor trap design can decrease trap efficiency regardless of bait. Several trap designs using females as baits attracted a large number of males but were not efficient in capturing them. However, when the trap design was altered by the addition of an entrance port closer to the males' searching surface, in this case the bark of the tree, the trap efficiency was doubled. This modification eliminated extended search by the male as a prerequisite to capture. This increase in efficiency was also noted in tests using wicks containing racemic disparlure as bait in place of virgin female moths.

Although vision has been implicated in male sexual behavior (Brown and Cameron 1977, Richerson 1977), trap color does not appear to affect trap efficiency. Holbrook et al. (1960) tested several different colors of traps, but statistical analysis of trap catches revealed no significant differences. More recent studies (Paszek and Tardif 1974) using racemic disparlure-baited traps have confirmed these results.

Determining the most effective height at which to place traps has long been the object of research. Forbush and Fernald (1896) reported that traps placed closer to the ground captured more males than traps placed higher in trees. Collins and Potts (1932) found that traps placed at 21.3 m in height did not capture any males; Holbrook et al. (1960) recorded little effect on the number of males captured when traps were placed anywhere between ground level and 1.8 m in height, but capture was reduced in traps placed at 3.6 m. Racemic disparlure-baited traps placed at 1 m above the ground caught more males than traps placed at 4–5 m (Beroza et al. 1973, Stevens and Beroza 1972), and Granett (1974) reported higher catches at 0.5 m and 1.5 m than at 3 m or 10 m. Hall et al. (1974) found that traps placed at ground level and at 1 m captured significantly more males than traps at 2 m, 3 m, and 6 m, while Cardé et al. (1975b) found that traps from 10–20 cm high captured significantly more males than traps hung directly above them at 1 m in height.

The behavioral reasons for differential trap catch at various heights have been explored only recently. Richerson et al. (1976a) demonstrated that males tend to restrict their searching activities to the height of the highest pheromone source. In these studies, they found that when traps were placed at 2 m in height, males were captured on unbaited sticky panels at 2 m but not higher. In dense, natural populations, Richerson et al. (1976b) noted a large portion of the

male searching activity was below crown level (4 m). A more complete explanation is still needed to characterize how population density affects trap efficiency. There is evidence that male searching behavior in different population densities may be altered.

Trap capacity may alter trap efficiency. If a sticky surface is used to retain captured individuals, a point is reached where a trap begins to fill with moths, and the trap begins to decrease in efficiency. Holbrook et al. (1960) recognized this as did Cardé et al. (1977c). Granett (1973) designed and tested a large capacity trap that could be used for monitor trapping. Detection traps should be designed to maximize efficiency for capturing one or, at most, a few moths. However, when designing traps for monitoring purposes, capacity must be taken into consideration. It is even possible that a trap designed with decreased attractiveness or efficiency might be considered. Currently, work is underway to maximize trap efficiency to improve detection and delimitation surveys. The delta trap (figure 6.4–3) baited with the (+) enantiomer of disparlure is the most sensitive detection and survey tool now available. As new bait dispensers and design modifications are incorporated into operational programs, traps will become even more valuable for detection and should fill a need for monitoring and other research purposes as well.

Male Dispersal

A knowledge of gypsy moth male dispersal behavior is of prime importance in establishing criteria for a pheromone-baited trap survey and detection or monitoring program. The efficiency of a system will depend not only on the "effective zone" of a trap but also on the behavior of dispersing males and the distances they may fly. This knowledge is also needed by investigators dealing with mating disruption and with sterile male studies.

Male flight is only one of many components of gypsy moth behavior that is poorly described. Longevity, flight periodicity, flight ability, and behavior will, along with other factors, determine the

Figure 6.4–3.—*Delta trap.*

total distance a male may cover. Males usually initiate flight within a few hours of eclosion. However, these initial flights appear to be of only short duration and males soon settle in sheltered locations, such as the underside of leaves and stems. After some period, males initiate flight again and begin actively searching for females. Recent studies indicate that the majority of males (around 80 percent) do not locate pheromone sources until the first full day of adult life—that is, the day after eclosion.

The diel periodicity of male response to pheromone is described by Cardé et al. (1974), who reported that the period of greatest response was between 1100 and 1500 hours; an additional response period can occur at dusk when evenings are warm. Male flight periodicity probably occurs with the same rhythm. Recent studies confirm that male sexual activity periodicity is modified by environmetnal factors. On warm days, a large amount of male activity may occur before 1000 hours. Cooler days delay most activity until afternoon hours. Longevity of males in the field will also affect the distance they can disperse. Collins and Potts (1932) reported they never captured males longer than 4 days after release. Collier and Downey (1965) found they rarely recaptured males after 3 days. In recent studies, the majority of males were found to be most active on the first day of adult life; activity decreased steadily thereafter. When males were 4 days old, few responded to either natural or synthetic pheromone sources.

Initiation of flight was at one time thought to be in response to some threshold concentration of pheromone (Schwinck 1958). Recently, Cardé (1977) demonstrated that males initiated and maintained flight in the absence of any pheromone sources; these flights were generally oriented at an angle to the windflow. Doane (1968) had noted that males in nonoriented flight were flying at some slight angle to the wind direction. Schwinck (1958) described such flights as "long-distance unoriented runs." The distance a male may cover in these long-distance runs over a lifetime is still not known.

Early attempts to quantify the distances over which males were attracted to pheromone sources indicate a trap's "effective zone" may be small. Collins and Potts

(1932), when using traps baited with virgin females (12–20), reported that males were rarely capable of flights of 3.7 km, and generally males were not recovered further than 800 m from a release point. The proportion recovered even at this distance was small. It was noted, however, that a few males were capable of covering long distances in short periods of time—for example, 1200 m in 55–105 minutes. Holbrook et al. (1960) found that released males sometimes were attracted up to 800 m to traps baited with extracts of female tips, but with more regularity to traps 536 m from the release point. Again, the percentages of released males recovered were small.

Paszek (1974) noted that traps baited with 50 female moths (4–48 hours old) only attracted 7.5 percent of males released 160 m downwind from them. Using (±) disparlure-baited traps with males released in the four cardinal directions at various distances from the traps, Paszek attempted to quantify the "effective zone" of a trap. The results over a 3-year period are consistent despite changes in trap design and bait dispensers; delta, Johnson, and cylinder traps were included in these trials. It was found that, as distance from the trap to the release point increased, trap catch generally decreased. At 1,609 m, recovery averaged about 0.13 percent, at 1,207 m about 0.07 percent, at 804 m about 0.62 percent, at 402 m about 0.73 percent, at 201 m about 2.5 percent, and at 100.5 m about 2 percent. The closest release point used was 50 m and recovery was about 1.5 percent of males released. The increase in the proportion of males recovered at 201 m and 100.5 m is still unexplained.

Recently, monitoring of male dispersal was attempted. Tests were conducted in uninfested circular plots of various diameters, and delta traps, baited with racemic disparlure, were placed on the circumference of these plots at a 30.5 m spacing. One-day-old marked males, eclosed from field-collected pupae, were released from the center of plots in groups of 100. A separate plot was used for each trap distance, and a total of 10–11 releases was made in each plot. The proportions of males recovered for all trap-to-release-point distances were small, but they steadily decreased (with one exception) with increased

distance the male had to fly (figure 6.4–4). The percentage of males recovered in traps 88 m from the release point was larger than for those released only 22 m from traps. This anomaly cannot be explained except that, possibly, increasing trap density may have affected the behavior of dispersing males. The attractiveness of racemic disparlure as a bait in traps is lower than the (+) enantiomer, and its use in these trials could have resulted in depressed trap catches—that is, males may have been dispersing in larger numbers but simply were not captured. However, Schwalbe (Disparlure-Baited Traps for Survey and Detection, this chapter) describes a test comparing

traps baited with either (+) disparlure or the racemic mixture at intertrap distances of 805 m. He reported similar recapture rates for traps baited with both types of lure at this trap spacing.

It remains to be determined how far males disperse and how factors such as female density and environmental influences affect this dispersal behavior. The distance that a male orients directly to a pheromone source also must be known before the most efficient trapping schemes can be formulated.

Population Monitoring

Development of techniques for managing gypsy moth populations has been one of the major objectives of the Expanded Gypsy Moth Program. Management of an insect requires, among other things, a knowledge of the abundance of a population and changes in density which are occurring. Currently, most estimates of gypsy moth population density are determined from sampling egg masses; damage estimates are obtained from aerial defoliation surveys. Traps may offer an efficient and an effective means to monitor insect density, as has been done, for example, by Hochmut et al. (1977) for the nun moth (*Lymantria monacha* L.) in Europe. Currently the only operational use of pheromone-baited traps in the gypsy moth program is for detection and delimitation of populations outside of the generally infested area.

Accurate information about density and population trends, were it available, would enable managers to make decisions on application and timing of control strategies. The possibility of using pheromone-baited traps to monitor insect population density has been demonstrated by several workers: Hopkins et al. (1977) with the boll weevil, *Anthonomus grandis* Boheman; Riedl and Croft (1974), Riedl et al. (1976), Madsen and Vakenti (1972, 1973), and Culver and Barnes (1977) with the codling moth, *Laspeyresia pomonella* (L.); and Madsen and Peters (1976) with *Archips argyrospilus* (Walker). These monitoring techniques have been used successfully to time the application of control practices and thereby reduce the volume of chemicals used, number of applications, and costs involved.
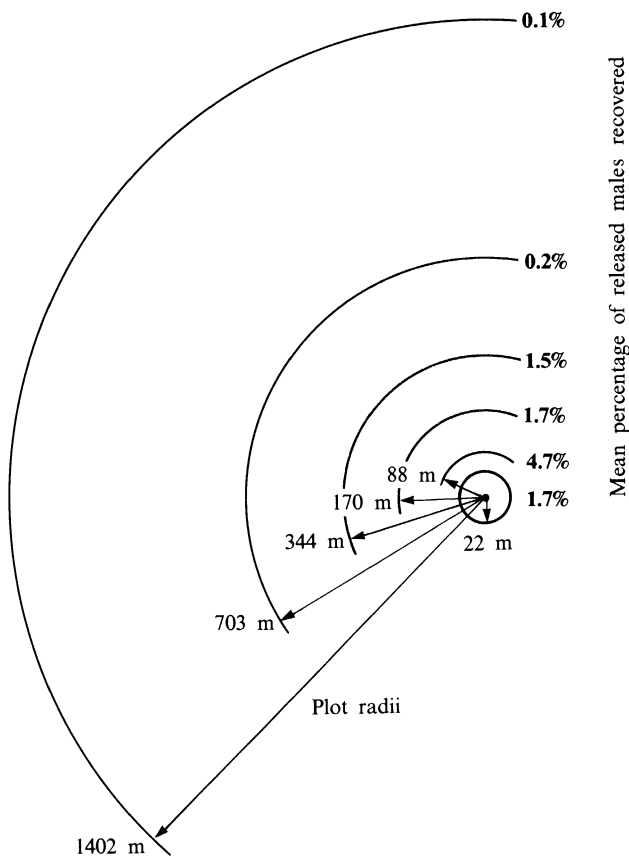
Figure 6.4–4.—*Mean percentages of released male gypsy moths recovered in uninfested circular plots of various diameters. Intertrap spacing 30.5 m for all plot diameters.*

Recently, it has been suggested that traps may be useful for predicting the potential for gypsy moth spread along the "leading edge" of the generally infested area. From a pest management viewpoint, areas found to have high insect densities would be treated with chemicals to reduce potential larval dispersal. Pheromone-baited trap captures reflect male moth density. Granett (1974), using pheromone-baited traps, found a significant correlation among pupal density, male mating potential, and trap catch in studies in Connecticut. In Pennsylvania a significant relationship was found between male trap catches and pupal counts in a lower population density than Granett's, but it was not possible to demonstrate a correlation between the numbers of males captured and postseason egg-mass counts.

The possibility of using pheromone-baited traps for gypsy moth monitoring purposes is still to be determined. Much more research and development are required to demonstrate that a trapping system can estimate density and predict change. Many factors may complicate development of a trap system—for example, trap efficiency may change with population density. Competing pheromone sources could lower trap efficiency—for example, as the ratio between females and traps increases, traps may become less efficient (Howell 1974). On the other hand, traps may become more efficient as populations increase because of increased sexual activity due to higher pheromone levels. When developing traps for estimating postseason egg-mass density, additional factors will have to be considered. For example, differences in male-to-female sex ratio due to differential larval and pupal mortality have been reported, and the relationship of trap catch in these cases would not be the same as in populations where the sex ratio is 1:1. Despite the difficulties described above, the potential benefits of developing traps for monitoring purposes and using them as part of an integrated management program would appear to warrant continued efforts in their development. In part because of the recent positive finding in the development of traps (including the identification of the (+) enantiomer as a more powerful attractant and continued efforts to develop better dispensers) and in

part because many variables of male behavior (including dispersal and mate finding in different population densities) are poorly described, the development of a monitoring trap has been delayed, and for practical purposes knowledge about using traps as population density estimators is lacking.

The Use of Disparlure to Disrupt Mating

Early Studies

E. Alan Cameron

Introduction

In addition to using disparlure in traps for the various purposes outlined in the previous section, it has been suggested by various authors that broadcast applications of the sex attractant might be used to disrupt chemical communication between the sexes (see for example Babson 1963, Beroza 1960, Beroza and Knipling 1972). Large sums of money, both prior to and during the gypsy moth program, have been earmarked and spent to test various approaches to gypsy moth control on the theory that disruption of chemical communication between adult moths would, as a consequence, disrupt and perhaps totally prevent their mating.

The mechanism by which disruption occurs is still not fully understood, although several possibilities have been suggested. Most of the early tests went on one of two explicit or implicit assumptions: That enough pheromone could be released into the atmosphere to adapt the males, thus precluding any response to pheromone released by females, or that enough individually attractive points (such as cork particles or microcapsules containing disparlure) could be established in the field, each as competitively attractive as a virgin female moth, to exhaust males by misdirected search and investigation of large numbers of false lures. Or it may be that females are capable of monitoring the ambient pheromone concentration and do not release their own attractant if a certain level is exceeded. Perhaps an artificially high atmospheric concentration of pheromone causes an

abnormal behavioral response such that overstimulation of males precludes finding females because of interference with normal response orientation (see for example Cameron 1974, Granett 1976).

If any of the foregoing mechanisms is correct, additional assumptions may also be required if control through behavioral disruption using disparlure is to succeed. For example, if males are misdirected to nonfemale attractive points, they must not "learn" to recognize, and subsequently avoid, such points; adaptation of receptors is a continuing and not a transient phenomenon; a visual response of male to female does not play a major role in mate finding; and there is not one or more additional, possibly but not necessarily synergistic, chemicals playing a major role in mate finding. Only through increased understanding of adult chemical communication and associated behavior will the needed knowledge be approached. Cardé (Precopulatory Sexual Behavior of the Adult Gypsy Moth, this section) reviews and summarizes this facet of gypsy moth chemical ecology.

Tests in Simulated Populations

The first application of disparlure in the field in quantities and in a manner intended to disrupt or confuse male moths was made in April 1971, on Dauphin Island, Ala., under the direction of F. M. Philips, U.S. Department of Agriculture, and E. A. Cameron (Cameron 1974). One 16-ha plot was sprayed with 62.5 mg lure per hectare in a mineral oil keeper and diluted into xylene; a second plot of the same size received 20,000 0.635-cm squares of hydrophobic paper, each containing about 2.5 μg disparlure (=50 mg disparlure per hectare). Johnson traps, baited with 10 μg disparlure in trioctanoin as a keeper and on a cotton wick, were deployed throughout the plots and untreated check plots to recapture males released within the plots. It was believed that such a bait equaled or exceeded the attraction of a virgin female moth (Beroza et al. 1971*b*), and that if recapture was reduced in treated plots, this would indicate the likelihood that males could not find and mate with females in a natural population of similar density. No males were

recaptured in traps in the plot treated with hydrophobic paper for 25 days after treatment; males were observed from time to time actively investigating and apparently attempting to copulate with individual pieces of hydrophobic paper.

In a second set of preseason tests in 1971, Stevens and Beroza (1972) obtained similar encouraging results when they distributed lure-impregnated hydrophobic paper over plots on Cape Cod, Mass., and used traps baited either with disparlure or with laboratory-reared virgin female moths as monitors. However, they recognized that persistence of lure activity was a formulation problem to be overcome if mating (interpreted from reduced recapture of males) were to be depressed over an entire flight season.

Beroza et al. (1973) revisited Dauphin Island for more preseason tests, this time applying disparlure at 8.2 g AI per hectare on 6–12 mesh granular cork and at the same rate in a molecular sieve formulation. Monitoring for recapture of released males was again done with Johnson traps baited with 10 μg disparlure in 2 mg trioctanoin keeper on a cotton wick. The formulations were effective in reducing male recapture for 7 weeks and about 1 month, respectively. In other preseason tests on Cape Cod, they tested a microencapsulated formulation of the lure as well as two cork formulations, and in postseason tests on the Cape the molecular sieve was tested against three different microcapsular formulations and granular cork. Depending on the test, lure-baited or virgin female-baited traps were used for monitoring results. Again, results were considered encouraging with both cork and microcapsular formulations. The authors did caution, however, that all of these tests had been conducted with laboratory-reared insects. Richerson (1972) and E. A. Cameron (1973) had already noted that there are pronounced differences in pheromone release and sexual behavior between laboratory-reared and wild adults, information more fully documented in their 1974 paper, and that validation of results of various tests in which populations were simulated with laboratory-reared insects was required using field-collected insects.

During the normal gypsy moth flight season in 1972 in central Pennyslvania, E. A. Cameron (1973)

conducted tests in much larger blocks. Disparlure-impregnated granular cork was applied at 7.5 or 25 g AI per hectare and wild insects were used in simulated populations in uninfested areas as monitors. Reduction in mating success was achieved in treated plots, but mating was still considered to be too high to cause a reduced population in the next generation. In another test, in which microencapsulated disparlure was applied at 5 g per hectare and results were monitored with laboratory-reared insects, no mating was recorded in treated plots. Similar tests in 1973, in which microencapsulated disparlure was applied at rates ranging from 2.5 to 15.0 g per hectare, continued to give encouraging results in that mating success of wild insects in treated plots was significantly reduced over the controls (Cameron et al. 1974, Schwalbe et al. 1974). But these tests also demonstrated that there were substantial behavioral and physiological differences between laboratory-reared and wild insects (Schwalbe et al. 1974).

Olefin Precursor of Disparlure

Beroza (1967) recognized a nonpersistent inhibitor of the gypsy moth sex attractant in extracts of the insect, later identified by Cardé et al. (1973) as 2-methyl-cis-7-octadecene, an olefin evidently the biosynthetic precursor of disparlure. Cardé et al. hypothesized that attraction to the pheromone is inhibited because the olefin interacts with the same receptor sites on the antennae of the male. Tests were designed in 1973 to examine the potential of this material, in microcapsular formulation, to disrupt mating of the gypsy moth. Traps baited with virgin female moths were used to monitor preseason and inseason tests on Cape Cod, where the olefin was applied at 61.75 g per hectare, while adults emerging from field-collected pupae were used as monitors in plots in central Pennsylvania where populations were simulated and where the material was applied at 15 g per hectare (Cameron et al. 1975). Based on the results, they concluded that the "... prospects of using the olefin as a substitute for disparlure for disruption of chemical communication in the gypsy moth are not promising."

Tests in Natural Populations

While most of the early tests of disparlure for disruption of mating were conducted in simulated populations, a series of empirical experiments was conducted in a natural infestation in summer 1971. Hydrophobic paper, impregnated with disparlure and a mineral oil keeper, was distributed over plots believed to have light or moderate populations. A total of 0.2 g disparlure per hectare was applied; a plot with heavy infestation received 0.4 g per hectare. At the same time, 6–12 mesh granular cork was applied with disparlure at the same rates to another set of similar plots. One plot received a treatment of disparlure in filter paper at a rate of 2.5 g lure per hectare. Mating and egg laying were observed in all plots; males were readily captured in monitor traps baited with 10 μg disparlure; postseason egg-mass counts ranged from 1,330 to 23,305 per hectare. Obviously no effective mating disruption was achieved (Cameron 1971, in part).

In the wake of these results, it was clear that further testing in natural infestations was premature until much more information on adult behavior was available. No disruption tests were conducted in natural infestations in 1972. Subsequent tests are discussed in the next section.

Disruption in Areas of Established Infestation

E. Alan Cameron

As available formulations of disparlure were improved and additional knowledge of moth behavior was acquired, various workers conducted tests with the pheromone in naturally infested forested areas. With few exceptions, the populations in test areas were sparse, a fact that greatly complicates assessment of results and that dictates that measurement of the extent of disruption of mating of the resident population must be supplemented with additional means of evaluation. Most often, these supplemental evaluation tools have included placement of virgin female moths and/or disparlure-baited traps at known locations within test blocks.

It has been generally accepted that mating disruption, if it can be manipulated by man, is most likely to occur in sparse populations. Theoretically, this method of control would work in an inversely density-dependent manner, increasing in its effectiveness as the insect population is reduced to lower and lower levels. Sparse populations of the gypsy moth may be encountered in incipient populations remote from the generally infested areas; in areas where defoliating populations have not yet built up; along the "leading edge" of the general infestation as it expands north, west, and south from the Northeastern United States; in areas where a population collapse has occurred as a result of various naturally occurring biological agents, such as NPV, parasitoids, and predators; or where populations have been reduced by the use of pesticidal sprays. Numerically similar populations may exist as a consequence of any of these situations. In terms of "population quality," however, these various populations could be quite different. We are barely scratching the surface in gaining knowledge of population quality, and this factor has been ignored for practical purposes in tests of the use of disparlure for mating disruption in established infestations.

Racemic disparlure, in one or another formulation, has been tested in all of these kinds of areas of established populations. In each test, the stated or implicit objective was to reduce mating to a degree that the population in the following year would be lower than that of the current year and certainly lower than would have been the case in the absence of treatment. For convenience of presentation, results of tests conducted along the "leading edge" of the expanding gypsy moth infestation and of tests conducted following pesticidal application to the larval stage are discussed later in separate sections. Tests conducted in sparse populations in Massachusetts, and designed to duplicate methods and verify results from tests along the "leading edge" in Maryland, are also included in the next section.

Cameron (Disparlure-Baited Traps for Population Reduction or Eradication, in chapter 6.4) has described the results of 2 years of study in which disparlure-baited traps were dropped in 1971 and 1972 over an isolated infestation in southwestern

Pennsylvania. Because of promising results with the newly developed microencapsulated formulation in 1972 (E. A. Cameron 1973), a decision was made to treat that infestation in 1973 with a broadcast application of the lure at a rate of 5 g per hectare. One week after the first pupae were observed in the field, approximately 32.8 km^2 of forested land were sprayed. The effect of the lure application was monitored by using disparlure-baited tube traps, by flagging and monitoring individual naturally occurring pupae and adults where possible, and by examination of resultant egg masses and postseason egg-mass counts.

Natural mortality during the larval and pupal stages decimated the already sparse population in the test area. However, available data indicate that only 16 of the 35 monitored females that emerged laid eggs. Postseason egg-mass counts were reduced by 88 percent compared with preseason counts, although much of this population reduction resulted from the mortality of immature stages and not the effects of the disparlure treatment alone. Only one male moth was captured in 650 traps. While it was not possible to monitor a similar isolated infestation as a control, results from tests being conducted elsewhere in simulated infestations of approximately the same population density suggested that the disparlure application did cause some disruption of mating success. Because funds ceased to be available to continue tests in that isolated infestation, no work was done past 1973. A population has persisted in the general area but has never reached defoliating levels.

A similar test was conducted the same summer in Massachusetts. Beroza et al. (1974b) sprayed 60 km^2 with microencapsulated disparlure at a rate of 5 g AI per hectare and used three separate areas of unspecified size and location as untreated controls. Results of the test were monitored using several criteria: A comparison of preseason and postseason egg-mass counts; capture of male moths in traps baited either with 10 μg disparlure plus 2 mg trioctanoin, or with wild or (later in the test) laboratory-reared virgin female moths; and mating of untethered females placed on tree trunks and recovered 3 days later. Following recovery, female

moths were dissected and examined for presence of sperm, and associated egg masses were held for 3 weeks and examined for embryonation.

Capture of male moths in traps was reduced by over 97 percent in lure-baited traps and over 99.9 percent in female-baited traps. Mating of untethered females was suppressed, particularly during the first 2½ weeks of the test, but increased markedly as male emergence peaked and the effect of the treatment presumably was reduced with aging. Postseason egg-mass counts were higher than preseason counts in treated plots but showed a significantly lower rate of increase than counts in the untreated plots. Beroza and coworkers noted that 15–17 percent of the egg masses from treated plots contained infertile eggs, presumably the result of females near death depositing eggs regardless of whether or not they had mated.

In 1974, microencapsulated disparlure was tested again in Massachusetts, this time to investigate the effect of increased rates of application. Three plots, each 2.6 km² and located within the 60-km² area treated in 1973, received one of the following treatments: 5 g lure per hectare, 20 g lure per hectare in one application, or 20 g lure per hectare divided between two applications of 10 g lure per hecatre each and applied 19–20 days apart (Beroza et al. 1975a). Results were monitored in a manner similar to the 1973 tests, except that Hercon® wicks were used in lure-baited traps. Even though mating success was only 31 percent in the untreated plot, mated females (both wild and laboratory-reared insects were used in this test, too) and trap catch were reduced by 47 and 55 percent, respectively, in the plot receiving 5 g lure. In the plots receiving the greater amounts of lure, these reductions were 94–97 percent in mating and 82–88 percent in trap catch. Egg-mass numbers were too low to analyze with any degree of confidence in this naturally collapsing infestation.

Granett and Doane (1975) applied microencapsulated disparlure in 1974 to a series of 1-ha circular plots in Connecticut using a knapsack mist blower. In these several test areas, preseason egg-mass counts were estimated at 0, 40 and 1,470 per hectare. Lure was applied at 18 g per hectare in a single application just after first adult emergence on the sites with low and high egg-mass counts, and just before adult emergence and again 3 weeks later in the plots with 40 egg masses per hectare. Disparlure-baited (20 μg plus 3 mg trioctanoin) traps and virgin wild female gypsy moths were used to monitor mating disruption. Trap catch was reduced by more than 98 percent when compared with catch in the untreated plots, regardless of preseason egg-mass counts, and none of the females deployed in any of the treated plots was mated, compared with 74 percent mating in the untreated plots. They concluded from their tests that disparlure has promise in reducing dense populations of the gypsy moth. However, the results remain anomalous because no one, including themselves (Granett 1976), has been able to duplicate the results in high populations.

One assumption that seems to have been made by the various teams working with disparlure to disrupt gypsy moth mating is that results obtained from monitoring devices—for example, baited traps or virgin moths, placed on the lower 2 m (often at about 1.5 m) of a tree—are indicative of what happens throughout all the various levels of the forest from the ground to the top of the canopy. What was actually happening higher in the trees was not monitored, largely as a matter of expediency. Richerson et al. (1976b) had noted that females tethered at 2 m on a tree trunk in pheromone-treated plots were mated much more successfully than those tethered in the litter. These same authors (1976a) reported that males were captured on sticky panels in equal numbers at 2, 4, or 6 m in plots which had been sprayed with disparlure but almost exclusively on the panels at 2 m only in untreated plots in which baited traps were deployed at 2 m. (In the absence of traps and treatment, no males were captured on the panels.) This would suggest that the vertical range of male activity is altered significantly by the presence of disparlure in the forest.

In 1976, tests were designed specifically to assess and compare results of mating disruption tests when the monitor organism was placed at the conventional

breast height (around 1.5 m), and at about 10 m up the bole of the tree. Tethered virgin female moths, which had emerged from field-collected pupae and were 1–2 days old when placed in the field, were deployed every second day throughout a 47-ha plot in central Pennsylvania that had been treated with microencapsulated disparlure at a rate of 20 g AI per hectare. After 2 days of exposure, females were recovered and dissected to determine if sperm was present in the bursa copulatrix or the spermatheca; any eggs laid were collected, held for at least 6 weeks, and examined for embryonation. If sperm was found in the female and/or embryos developed, the female was considered to have been mated. Results are presented in table 6.4–8. There was no difference in mating success at the two heights in the untreated plot, but there was a very highly significant difference in the treated plot. Proportionately much more mating was done by the females placed higher on the bole. Mating was suppressed in the treated plot, although not nearly enough to cause a population reduction. In fact, egg masses increased from a count of 41 per hectare preseason in the treated plot to 1,864 per hectare postseason (an increase of over 45×), and from 96 per hectare to 2,738 per hectare (over 28.5×) in the untreated plot (Cameron 1977 in part).

Similar tests were conducted in 1976 by others in eastern Pennsylvania, but technical difficulties and the mixing of laboratory-reared and wild females as monitors confounded the results, and a full report is not available.

Results obtained from this test are in accord with what would be expected on the basis of significantly reduced concentrations of disparlure in the forest at increasing heights above the ground (Plimmer et al. 1978). The same phenomenon, on a much smaller vertical scale, has also been reported by Caro et al. (1977) for disparlure concentration in the air above a grass field to which it was applied. One conclusion that could be drawn from these studies is that the microcapsules containing disparlure were not likely to be adhering to the foliage throughout the weeks following application and during the time the tests were executed.

Tests were designed for 1977 in which disparlure would be released from hollow fibres in plots 1 ha in size and circular in shape. An area supporting a sparse infestation in central Pennsylvania was chosen for these tests. Strips of 50 Conrel fibres, 20 mil i.d., were stapled to the lower 10–12 m of 250 trees in each treated plot, to provide 325,000 fibre ends per plot. Fibres were charged with racemic disparlure and about 30–35 percent hexane and were expected to emit 125 g lure per hectare over a period of 6 weeks. Tethered feral virgin females were used at 1.5 and 10 m, as in 1976, to monitor the effect of the emitted disparlure on mating disruption. Season-long results are presented in table 6.4–8. When data are examined on a day-by-day basis, one notes that, as the season progressed and more males were present in the plot, mating disruption was less effective in the treated plots; disruption increased again late in the flight season when fewer males were available. This pattern has been seen consistently over the years in similar tests.

One other point should be noted. There was no difference in mating success in the treated plots of females placed 10 m up the bole when compared with those at 1.5 m. And the proportion mated was remarkably similar to the proportion mated in 1976 tests at 1.5 m (table 6.4–8). Apparently the individual placement of fibre strips on the tree boles insured the presence of adequate lure to cause some mating disruption at least as high as was tested (Cameron 1977).

Problems remain with this system, however. Chemical analysis of a representative sample of fibres after the end of the season indicated that only about 5–10 percent of the lure was emitted (Bierl 1977), or perhaps about 6–12 g per hectare during the entire season. So it is still an open question whether a much larger rate of emission of racemic disparlure would increase mating disruption. Nor is there any evidence of any reduction in density of the natural population. Egg-mass counts in this sparsely infested area increased from about 26 per hectare preseason to 182 per hectare postseason (an increase of 7×), with no measurable difference in treated or untreated plots.

Table 6.4-8.—*Mating success of tethered female gypsy moths, central Pennsylvania, 1976 and 1977*

Female height[1]	Treated			Untreated			X^2 [3]
	Mated	Not mated	Percent mated	Mated	Not mated	Percent mated	
			1976[2]				
High	122	73	62.6	126	5	96.2	[3]48.66
Low	160	195	45.1	350	13	96.4	[3]229.97
X^2		[3]15.42			0.015		
			1977[4]				
High	280	345	44.8	539	41	92.9	[3]320.09
Low	271	374	42.0	596	37	94.2	[3]398.07
X^2		1.002			0.753		

[1]High: females tethered to the tree bole about 10 m from the ground; low: females tethered at 1.5 m.

[2]One plot each for treated and control; plot size 47 ha. Microcapsular formulation of disparlure containing 20 g per hectare applied by air.

[3]$P < 0.005$

[4]Three plots each for treated and control; plot size 1 ha. Data from plots pooled since there were no significant differences among replicates. Disparlure dispensed from 325,000 hollow fibre ends per plot intended to yield 125 g per hectare. This yield was not attained.

Source: Cameron 1977.

No tests similar to any of the ones described above have been conducted in which (+) disparlure has been used instead of the racemic mixture. The quantities of (+) disparlure that would be needed have simply not been available for testing. It remains to be seen if this material, which is so much more attractive than the racemic mixture (Cardé et al. 1977*b*, Plimmer et al. 1977*a*), would exhibit a similarly inceased level of mating disruption if applied in broadcast form. Such tests must await a breakthrough in chemical synthesis to permit production of at least kilogram quantities of the material.

Disruption Along the "Leading Edge" of the Infestation

Ralph E. Webb, Charles W. McComb, Jack R. Plimmer, Barbara A. Bierl-Leonhardt, Charles P. Schwalbe, and Robert M. Altman

Overview

Tests leading to this program have been summarized in this section (Evolution of Formulations, Early Studies, and Disruption in Areas of Established Infestation). Beroza and Knipling (1972)

suggested that the mating disruption technique was a density-dependent control strategy most effective in areas with low population densities. They hypothesized that this concept would be useful in two situations: Eliminating gypsy moth populations isolated from the main pest population centered in the Northeastern United States, and retarding the spread of the gypsy moth by elimination of sparse populations along the "leading edge" of the expanding population.

This technology, if demonstrated reliably to cause high levels of mating disruption, would have several uses along the "leading edge." It could be used against isolated pockets of gypsy moths to eliminate such foci before they built and spread—alone if the population was quite sparse, or in combination with pesticides against "hot spots." Moreover, it could be sprayed over large areas ahead of the advancing front, again in combination with pesticides used against identified "hot spots," to form a barrier that would retard further advance. Otherwise, reliance on retarding gypsy moth spread must rest on the wide-area application of traditional pesticides, with attendant public concern. Since all tests indicate that disparlure is innocuous against nontarget organisms, including

man, it could form the central part of a program using limited amounts of traditional pesticides that should preclude this public concern.

This technique will be used only experimentally until it can be demonstrated that it can indeed suppress a natural population. The degree of control needed for this depends upon population quality even more than population size—that is, a stable or declining population should be more susceptible to control by this technique than one that is increasing. Certainly any future model should take into account various rates of increase of population. No one can say at present, on the basis of the tests done to date, what percent of mating disruption is needed to suppress gypsy moth populations whatever the size or quality. Determining the degree of mating suppression needed to cause population reduction under the many conditions found on the "leading edge" will take several more years of careful experimentation. Meanwhile, it must be assumed that a rather high level (Beroza and Knipling 1972 arbitrarily chose 90 percent) of mating disruption must be the goal. A summary of work to date done on the "leading edge" follows.

Determining efficacy of disparlure as a mating disruptant in low-density areas posed a number of problems, most of which became apparent at the end of each summer's tests. Questions raised at the end of 1975 determined 1976 tests and more questions from 1976 guided 1977 tests; the process continues as this report is being written. Some of the questions have been answered, others are at this time still unresolved. This section will concentrate on general results, giving general experimental designs but referring the reader to companion technical publications for many specific details.

Cecil County, Md., is in many respects an ideal test area for the above concepts, because it has a low insect population density and is on the edge of the expanding gypsy moth population. It was precisely the type of area that theory predicts disparlure might be of value for retarding spread. Two types of tests were performed. The first series, conducted in 1975 and 1976, evaluated disparlure formulated in sprayable microcapsules. The second type, run in

1976 and 1977, evaluated disparlure released from plastic dispensers. Associated tests were done in Massachusetts in 1976 and 1977 to back up the Maryland tests.

In 1975, a large-scale pilot test was conducted to evaluate the ability of disparlure to disrupt mating over a large area of northeastern Maryland that had a general but sparse infestation of gypsy moth. One of the major unsolved problems was that no valid survey technique existed that could adequately measure the gypsy moth population level present in Cecil County at the time of these tests. Because it was important to have this information, attempts were made to assess population densities for each of the tests. The disparlure was applied in NCR microcapsules containing 2.2 percent AI, a formulation that had previously given promising levels of disruption against low-level gypsy moth populations in Massachusetts (Beroza et al. 1974*b*, 1975*a*).

Important objectives of this test were to gain field experience in the large-scale suppression of gypsy moth using disparlure, to test and refine techniques and to determine weakness in strategy or formulation that had not heretofore become apparent in less extensive tests.

The 90 percent reduction level postulated by Beroza and Knipling (1972) was not achieved in 1975. It is felt that this could be accounted for by weakness in the formulation; the sticker used with the NCR microcapsules in the previous Massachusetts studies (UCAR #1 Latex®) was not available for the 1975 test, and a different sticker, Rhoplex B-15®, was substituted. Laboratory tests in 1976 with candidate stickers (Webb et al. 1978*c*) demonstrated the unsuitability of Rhoplex B-15®, compared to UCAR #1 Latex® as a sticker for NCR microcapsules. This, combined with results from spray cards in the plots, indicated that the sticker failed to hold the microcapsules through the extensive rainfalls of that season. This could have reduced the pheromone titer in the air to a point where the concept did not receive a fair test. Hence, it is concluded that improvements in the formulation were needed.

During winter 1975-76, candidate formulations and stickers were evaluated in laboratory tests (Webb

et al. 1978c), and of these, six different formulations (with the most promising sticker added) were field tested against the 2.2 percent NCR microcapsules plus the Rhoplex B–15® sticker combination used in the 1975 pilot test. Duplicate tests were run in Maryland and Massachusetts to evaluate the formulations under different environmental conditions and population levels.

Although improvements were obtained with aerially applied microcapsules in the 1976 tests, loss of microcapsules still presented a problem, especially under conditions of heavy soaking rains when the microcapsules containing the pheromone tended to be washed away. Moreover, the first-order release characteristics of microcapsules made it inappropriate for certain basic studies thought necessary. (A first-order system releases the most pheromone at the beginning of the test, with the release rate declining over time. A zero-order system releases pheromone uniformly over time, a definite advantage for dose response tests.) Therefore, further tests in 1976 and 1977 evaluated the disruptive capacity of disparlure released from stationary plastic dispensers. Grids of Hercon® dispensers had been successfully utilized in mating suppression tests with other insect species (Mitchell et al. 1976), and such a grid approximated a zero-order release system over the life of a test.

1975 Pilot Test

Egg-mass surveys conducted in winter 1974–75 indicated that the northern portion of Cecil County would be a suitable area for the experiment. This survey turned up egg masses in 88 different locations scattered throughout the survey area. The treated area included approximately half of the known infested locations, leaving the areas containing the other infested locations as a control for the experiment. Results were evaluated by comparing egg-mass densities in both treated and control areas before and after treatment, comparing the number of male moths captured in disparlure-baited traps in treated versus nontreated areas, and comparing the mating success of sentinel laboratory-reared virgin females in treated and control areas.

Four hundred evaluation sites, evenly divided between treated and control areas, were selected. These sites included all 88 sites where gypsy moth egg masses were found, plus 312 sites set up at susceptible locations. (For details on site arrangement and methodology, see Webb et al. 1978a.) For many reasons, including logging operations at several sites, several sites had to be abandoned, so that the results were actually obtained from 188 sites in the treated areas compared with 183 sites in the control areas. A virgin female (1 day old or less) was placed at each site and replaced on a 2- or 3-day schedule for the duration of the test, then returned to the laboratory with any deposited eggs. Females were held until the completion of egg deposition, then examined for sperm by the technique of Stark et al. (1974). All eggs were subsequently checked for embryonation. The presence of sperm in the bursa copulatrix or embryonation of the eggs was taken as indication of successful mating. At each site a trap baited with a Hercon® wick containing racemic disparlure was placed 60 m from the female placement station, and the number of males captured in the treated areas was compared with the number caught in control areas. Disparlure was aerially applied June 23–25 over about 19,392 ha, with the amount of active ingredient actually sprayed ranging from 13.5 to 16.75 g per ha. The sprays were well applied on the second day (on about 8,080 ha), but problems occurred on the first and third days with the spray equipment so that coverge was not as uniform on those days.

Results reported here are the data from 13 evaluation periods beginning July 7, when the first native male activity was seen, and extending through August 7, after which time male flight had virtually ceased. For purposes of evaluation, the treated areas and the control areas were divided into five sectors each (Webb et al. 1978a). Of the five treated sectors, sectors 2 and 4 were uniformly treated, while sectors 1, 3, and 5 were less so because of difficulties with the spray equipment over these areas. Of the control sectors (sectors 6–10), sectors 7, 8, and 10 contained evaluation sites possibly contaminated by a small amount of pheromone during applications or, being contiguous to spray plots, were subject to drift of

pheromone after application. Sectors 6 and 9 were not flown over by spray planes and had little chance of pheromone contamination.

The data in table 6.4–9. are only from females for which both sperm data and egg embryonation data have been recorded. This amounted to 485 double determinations from treated sectors and 482 from control sectors. Of these, 11 percent were mated (positive for sperm) in the treated sectors versus 35 percent in the control sectors, indicating that considerable mating reduction occurred. In the treated sectors, mating ranged from 5.3 percent in sector 4 to 16.8 percent in sector 5. The lowest levels of mating occurred in sectors 2 and 4, which received the most uniform treatment, indicating that uniform application of the pheromone will be essential if pheromones are to be used in large field programs. In the control sectors, mating ranged from 30.3 percent in sector 8 to 38.1 percent in sector 9. Sectors 6 and 9, which had the least chance for contamination, had the highest percentage of mating.

Of 55 sperm-positive females from the treated sectors, only 25 laid eggs that embryonated; eggs from

the other 30 failed to embryonate, accounting for 54.5 percent sterility in eggs from inseminated females. This compared with 37 infertile egg masses (out of 168 inseminated females) in the controls ("sterility factor," 21.1 percent). When this "sterility factor" is included, effective matings (those yielding embryonating egg masses) amounted to 5.2 percent for the treatments versus 27.2 percent for the controls, or an effective control of 81 percent. It was also seen that section 9, which had the least possible chance of contamination, had the highest percentage mating and the lowest percent sterility of inseminated females. The "sterility factor" mentioned here was also seen in subsequent tests, discussed in more detail later in this section.

Mating success on different days (Webb et al. 1978a) had several irregularities that corresponded to periods of rainfall. The period just after application received considerable rainfall (5.5 cm for June 26–30). This provided a prolonged period of soaking with shorter periods of intense rainfall, conditions ideal to facilitate dislodging of the freshly applied microcapsules (Webb et al. 1978c). Total rainfall for July was 27.9 cm. Given the inability of Rhoplex B–15® to hold

Table 6.4–9.— *Percent of mating, sterility of inseminated females, and effective mating of gypsy moth females in areas treated with disparlure[1], 1975*

Treatment	Sector	Number of evaluation sites	Total females	Mated females	Percent mating	Sterile females	Percent sterility[2]	Percent of effective mating[2]
Treated with NCR 2.2%	1	39	131	13	10.0	9	69.2	3.1
	2	43	103	8	7.8	4	50.0	3.9
	3	20	51	7	13.7	6	85.7	2.0
	4	26	57	3	5.3	1	33.3	3.5
	5	60	143	24	16.8	10	38.5	9.8
Total		188	485	55	11.3	30	54.5	5.2
Untreated	6	59	156	57	36.5	16	29.1	26.3
	7	30	87	28	32.2	6	21.4	25.3
	8	26	76	23	30.3	4	17.4	25.0
	9	46	118	45	38.1	6	13.3	33.1
	10	22	45	15	33.3	5	33.3	22.2
Total		183	482	168	34.9	37	21.1	27.2

[1]Formulated 2.2 percent in NCR microcapsules.
[2]Data transformed by $\sqrt{\text{arcsine}}$ for analysis of variance. Treatment effects for percent mating and percent effective mating were significant at $P < 0.0001$, while effects for percent sterility were significant at $P < 0.025$.

microcapsules under this type of weather (Webb et al. 1978*c*), the importance of these weather factors cannot be overstated.

Results from the baited traps generally paralleled results for mating disruption. Overall, 128 males were caught in traps located in treated sectors, compared with 325 caught in traps located in the control sectors, for an overall reduction of 61 percent. However, 70 moths caught in the treated sectors came from one site, a "hot spot" at which 131 egg masses were found.

Results of the egg-mass evaluations were disappointing, with too few egg masses found in either the control or treated areas to permit a conclusion. (For details on trap and egg-mass evaluation, see Webb et al. 1978*c*).

1976 Spray Tests

Six candidate formulations found to have promising characteristics in a laboratory screening program (Plimmer et al. 1977*b*) were selected for evaluation in the field against the 1975 2.2 percent NCR microcapsules plus 1 percent Rhoplex B-15® sticker. A new sticker, Monsanto Rh-1645®, was found far superior to Rhoplex B-15® as an adhesive for microcapsules (Webb et al. 1978*c*) and was chosen as a replacement sticker for the new formulations to be evaluated. Field testing was done in both Maryland and Massachusetts to test effectiveness in different population densities and histories and under different climatic conditions. The test area in Massachusetts, in the Fall River State Forest, was characterized by a sparse, stable gypsy moth population, while the Maryland plots lay in Cecil County in the same general area as the 1975 tests. Thirty-six plots, 16 ha each, were established in each test area. Eight plots in Massachusetts were randomly selected for intensive egg-mass surveys (80 man-hours per plot) and for burlap banding (100 per plot) to monitor the development of the larval population. In Maryland, 800 burlap bands were similarly placed, but no egg-mass survey was done because of the high cost and low precision attendant to such surveys in sparse populations. (The 800 burlap bands in Massachusetts

yielded 88 pupae, while those in Maryland yielded 46 larvae. The population in Massachusetts was about two egg masses per hectare, the population in Maryland was estimated to be 1 egg mass per hectare or a bit less. Details of the spray operation and characteristics of the spray formulations are found in Schwalbe et al. (1979).

Mating disruption was monitored as in 1975, except that field-collected females (collected from moderately defoliating populations in New Jersey, Pennsylvania, New York, Vermont, and Maine) were used instead of laboratory-reared females. Laboratory-reared insects were used after the supply of field-collected females was depleted on July 29. At both locations females were checked for sperm, and the eggs associated with each female were checked for embryonation after 4 weeks. Formulations evaluated and results for these tests are given in table 6.4-10. The data are totals of all fertility determinations (sperm determinations and egg embryonation) performed during the test period. Mating in treated plots ranged from 8.2 percent (1976 2 percent NCR) to 40.3 percent (MGK) in Massachusetts, and from 1.3 percent (1976 2 percent NCR) to 44.4 percent (MGK) in Maryland. Mating in control plots averaged 56.1 percent and 53.0 percent in Massachusetts and Maryland, respectively. Ranking of the seven test formulations in order of effectiveness was similar in the two studies.

The degree of mating disruption was generally greater in Maryland tests where substantially lower populations of male moths existed, supporting a hypothesis that the ability of disparlure to disrupt mating is affected by male population density. It is useful to compare the 1975 2 percent NCR formulation with its 1976 counterpart. The improved performance of the 1976 2 percent NCR seen at both locations is probably due to two factors—decreased particle size (from 400 μ to 250 μ maximum diameter) and the improved sticker. Both factors should substantially improve the weatherability of the product (Webb et al. 1978*c*). The improvements should facilitate the practical utilization of sprayable formulations of disparlure for control of gypsy moths through mating disruption.

Table 6.4-10.—*Formulation tests: Incidence of mating of female gypsy moths placed in test plots, 1976*

Location and treatment	Number of sperm and egg-mass determinations	Number fertile[1]	Percent mated
Massachusetts			
1976 2% National Cash Register (NCR)	437	36	8.2
1975 2% National Cash Register (NCR)	358	54	15.1
1976 4.3% National Cash Register (NCR)	331	50	15.1
1976 10% National Cash Register (NCR)	322	64	19.9
Conrel	355	89	25.1
Stauffer	416	123	29.6
McLaughlin-Gormley-King (MGK)	462	186	40.3
Controls	725	407	56.1
Maryland			
1976 2% National Cash Register (NCR)	75	1	1.3
1976 10% National Cash Register (NCR)	149	7	4.7
1976 4.3% National Cash Register (NCR)	100	5	5.0
Conrel	76	7	9.2
1975 2% National Cash Register (NCR)	91	9	9.9
Stauffer	94	25	26.6
McLaughlin-Gormley-King (MGK)	108	48	44.4
Controls	232	123	53.0

[1]Determination considered fertile if either egg-mass or sperm determination found positive.

Wick Tests

The goal of the 1976 wick test was to adapt the use of pheromone dispensers to provide season-long, steady-state release of pheromone to inhibit gypsy moth mating success. Nine blocks (1 ha each) were established on relatively level terrain within wooded stands (mixed hardwoods containing a high percentage of red and white oaks) without cleared areas. Hercon® dispensers were placed in a grid pattern by stapling them to trees at 1.5 m above ground. Two dosage levels were evaluated: One consisting of 1,600 6.5-cm² dispensers per hectare (four dispensers at each of 400 sites on a 20×20 grid with 5-m spacing between grid points); the other with 200 dispensers per hectare (two dispensers at each of 100 sites on a 10×10 grid with 10-m spacing between grid points). Each treatment was replicated three times and there were three control plots. The three-layer Hercon® laminated dispensers (Beroza et al. 1975b) had a 3.3 mg per cm² disparlure concentration (12.5–13 percent by weight) and were 6.5 cm² by 10 mil thick. Measured emission rate (Bierl and DeVilbiss 1975) was 0.34 μg

per hour per wick at 32° C and a constant air flow of 100 ml per minute. It has been calculated that the actual amount of lure lost during outdoor aging is about six times as much as the amount indicated from the laboratory measured emission rate of aged dispensers.

Adult female gypsy moths (24 hours old or less) from field-collected pupae were exposed at mating stations established on a 3×3 grid (nine stations) in the center of each plot, 20 m apart and beginning 30 m from the perimeter. They were tethered (Richerson et al. 1976b) under tarpaper flaps placed 1.5 m up on the tree. Every 3 days the females were collected using the procedure of the previous tests, replaced, and returned to the laboratory where they were examined for the presence of sperm; their eggs were examined for embryonation as in previous tests. The test started July 14 and ended August 7, for a total of eight placement periods.

In 1977, two types of tests were run: Dose response tests and a spatial distribution test. The dose response tests were conducted at two locations chosen to

represent different population densities. One test was set in the Bridgewater State Forest, Mass., in predominantly oak woods with a moderate but subdefoliating population; a second test was located in sparse populations in Cecil County, Md. Both tests used 10×10 grids of dispensers with 10 m spacings (1 ha total area). Pheromone doses were controlled by the length of wick used at each grid point (20.3, 5.1, and 1.3 cm per grid spot in Massachusetts, and 5.1, 1.3, and 0.3 cm per grid spot in Maryland. This was equal to 800, 200, and 50 6.5-cm² wicks per hectare and 200, 50, and 12.5 6.5-cm² wicks per hectare, respectively). Different rates at the two locations were used because it was known from the 1976 data that 200 wicks per hectare were efficacious in Maryland, and it was desired to determine how low the efficacy point was.

In Massachusetts, with its higher population density, it was thought wise to use a higher dose range. It was assumed that the emission of disparlure from the wick was primarily from the surface of the wick, with little being emitted from the edge; thus it was believed that a sixteenfold emission rate difference existed at both locations. However, recent measurements indicate that a significant loss of lure occurs through the edge. Release rates at 32° C in a 100 ml per minute air stream for 2.5×0.3 cm, 2.5×1.3 cm, and 2.5×5.1 cm wicks have been calculated to be 0.13, 0.16, and 1.2 μg per hour. Thus, instead of a sixteenfold rate release difference in Maryland, it was actually about a tenfold difference. Little difference existed in the release rate of 2.5×1.3 cm and 2.5×0.3 cm wicks, probably because the amount of edge is about the same for both wicks. This was not as serious in the Massachusetts test, since the ratio of the edges of 2.5×1.3 cm, 2.5×5.1 cm, and 2.5×20.3 cm wicks approaches the ratio of the surface areas. All wicks were placed facing north at 1.5 m height. Results were compared with those from untreated control plots. Laboratory-reared females (Otis AFB F-16 strain) were used throughout. Females were replaced at the female exposure sites (nine per block on a 3×3 grid as in 1976) on a 2-day schedule. In Massachusetts, there were three replicate blocks per treatment, and only

egg embryonation data were obtained. In Maryland there were four replicate blocks, and both sperm determination and egg embryonation data were taken for each female where possible. Tests ran from July 5 to July 30 in Maryland and from July 20 to August 11 in Massachusetts. This was the approximate male flight period in these areas.

The spatial distribution test in Maryland was also run in 1-ha blocks, again using 10×10 grids of wicks with 10 m spacings. Treatments were: 200 wicks per hectare all placed at 1.5 m on the tree, 100 wicks placed at 1.5 m and 100 wicks placed on the same trees at 6 m, and untreated controls. In all plots, females were placed at each exposure site at both 1.5 m and 6 m. The test ran from July 5 to July 30, with females replaced every 2 days. There were four replicates per treatment. Again, both sperm determination and egg embryonation data were obtained for each female.

A clear dose response was seen only for the 1977 Massachusetts test (table 6.4-11). The reduced effectiveness of disparlure in preventing mating even at 800 wicks per hectare in Massachusetts, compared with the more favorable results obtained in the sparse populations in Maryland, argues strongly for the conclusion that mating disruption is an inverse density-dependent phenomenon. In neither year was a clear dose response seen in Maryland, indicating that even low doses may disrupt mating in sparse populations. The dip in effectiveness of the 50 wicks per hectare in Maryland was due to the poor performance of just one of the four replicates that was located at a "hot spot" of gypsy moth activity, which again indicates that this phenomenon is inversely density dependent. On the other hand, the performance curve was still rising at 800 wicks per hectare in Massachusetts, indicating that higher doses of pheromone might well suppress mating in higher populations. Such an event has been reported elsewhere (Granett and Doane 1975), although not with wicks.

Results of the spatial distribution test are given in table 6.4-12. Females placed at 6 m were mated at about the same rate as those placed at 1.5 m. Likewise, no differences were seen in mating reduction whether

Table 6.4-11.—*Dose response tests: Mating disruption effected by disparlure emitted from Hercon® dispensers. Sperm and egg-mass determinations from Cecil County, Md., July 1976 and July 1977, and egg-mass determinations from Bridgewater, Mass., July-August 1977*

Location and treatment (dispensers per hectare, by year)	Sperm Determination data[1]			Egg embryonation data[1]		
	Number of females determined for sperm	Number fertile	Percent mated	Number of egg masses determined	Number fertile	Percent mated
Maryland 1976						
1600	58	1	1.7	70	1	1.4
200	77	3	5.7	93	1	1.1
Control	53	32	60.4	79	57	72.2
Maryland 1977						
200	189	33	17.5	202	1	0.5
50	173	51	29.4	204	26	12.7
12.5	205	39	19.0	224	6	2.7
Control	194	116	59.8	265	151	57.0
Massachusetts 1977						
800	—	—	—	159	57	35.8
200	—	—	—	166	96	57.8
50	—	—	—	164	114	69.5
Control	—	—	—	173	166	96.0

[1]For analysis of variance, data were entered as percentages transformed by $\sqrt{}$ arcsine. For the Massachusetts data, treatment effects for percent of mating (egg embryonation) were significant at $P<0.0001$. For the Maryland data, treatment effects for percent of mating (sperm determination and egg embryonation) were also significant at $P<0.0001$.

Table 6.4-12.—*Spatial distribution test: Effect of placing pheromone dispensers at 1.5 m and 6 m vs. all dispensers placed at 1.5 m on the mating of female moths placed at 1.5 m or 6 m in the canopy[1], Cecil County, Md., July 1977*

Dispenser placement	Female placement	Sperm determination data[2]			Egg embryonation data[2]		
		Number of females determined for sperm	Number fertile	Percent mated	Number of egg masses determined	Number fertile	Percent mated
100 high 100 low	6 m	160	24	15	207	2	1.0
100 high 100 low	1.5m	140	20	14.3	182	3	1.6
200 low	6 m	192	33	17.2	214	2	.9
200 low	1.5m	159	28	17.6	176	3	1.7
Control	6 m	185	101	54.6	216	112	51.9
Control	1.5m	160	94	58.8	214	114	53.3

[1]Totals from four replicate blocks.
[2]For analysis of variance, data were entered as percentage transformed by $\sqrt{}$ arcsine. Treatment effects for percent mated (both sperm determination and egg embryonation) were significant at $P<0.0001$.

the wicks were placed at 1.5 m or were divided 50:50 between 1.5 m and 6 m. For more details and discussion, see Webb et al. (1978b).

Sterility Factor

As seen in tables 6.4-11 and 6.4-12, a pronounced discrepancy existed between the sperm determination data and the egg embryonation data in Maryland in 1977 in both the dose response test and in the spatial distribution test. In the dose response test, percent matings were 17.5, 29.4 and 19.0 (vs. 59.8 percent for the controls) for the sperm determination data for 200, 50, and 12.5 wicks per hectare, respectively, compared with percent mating of 0.5, 12.7, and 2.7 (vs. 57.0 percent for the controls) for the egg embryonation data. In the spatial distribution experiment, matings in the treated plots compared with the controls ranged from 14.3-17.6 percent (vs. 54.6-58.8 for controls) when only the sperm determination data were considered, but were 0.9-1.7 percent (vs. 51.9-53.3 percent for controls) when only the egg embryonation data were considered. It was this large discrepancy that indicated that a sort of "sterility factor" was somehow operating in the test.

In Table 6.4-13, the data for the three wick experiments done in Maryland are rearranged using only data collected from females for which both egg-mass data and sperm determination data are available. For simplicity, the data for the spatial distribution test reflect combined results for females placed at 6 m and those placed at 1.5 m. Females found positive for sperm in treated plots laid eggs that usually failed to embryonate, while egg masses from similar females from control plots usually did embryonate. The "sterility factor" ranged from 63.8 to 96.4 percent in the various 1977 treatments. Thus, although mating reductions based on sperm determinations ranged from 51 to 76 percent, when the "sterility factor" is considered, the effective mating reduction rises to 81-99 percent.

As discussed previously a "sterility factor" of about 55 percent was seen in the 1975 data (table 6.4-9). In table 6.4-14, the 1976 spray test data are reexamined for the presence of sterile egg masses obtained from

inseminated females. Such sterile egg masses were present from both locations but not in the numbers found in the 1975 or 1977 tests. This may have been because of the higher temperatures prevailing in 1977. The average high temperature for July was 31° C in 1977, with 12 days recording in the 32° C or above range. Temperatures averaged 29.5° C with 5 days over 32° C in 1975, and 29° C with only 2 days over 32° C in 1976. The moths may have been more stressed in 1977. On the other hand, laboratory-reared insects were used in 1975 and 1977, while field-collected insects were used in 1976. Tests have been designed for 1978 that will directly compare laboratory-reared versus native Maryland gypsy moth females for several factors, including the relative incidence of the "sterility factor" in the two female types. Other tests are visualized to elucidate this phenomenon. At present, several hypotheses have to be tested before the "sterility factor" can be fully explained.

Summary and Conclusions

In 1975, racemic disparlure applied in 2.2 percent NCR microcapsules at the achieved rate of 13.5-16.75 g AI per ha caused substantial mating suppression of laboratory-reared females over a large area (about 188 km²) compared with females placed in control areas. In the treated sectors, mating varied from 5 to 7 percent in sectors where spray cards indicated that a uniform application occurred, to as high as 17 percent in sectors where equipment malfunctions resulted in a less uniform application. In control sectors, mating varied from 30 to 33 percent in sectors adjacent to spray areas and subject to possible pheromone contamination, compared with 36-38 percent in areas farther from treated sectors. These trends indicate that the overall percent mating reduction figure may be conservative, and they also demonstrate the importance of precision in the spray operation for successful large-scale field programs. Soaking rains soon after application in June and torrential rains in July adversely affected results in 1975 because of the use of an unproven sticker that subsequent laboratory tests demonstrated would not

Table 6.4–13.—*Data from Cecil County, Md.: Dose response and high-low tests rearranged to demonstrate a "sterility factor" present in disparlure treated plots, July 1976 and July 1977*[1]

Location and treatment (dispensers per hectare) by year	Number of determinations	Number of females inseminated	Egg masses from inseminated females			Percent of fertile egg masses from total determinations[2]
			Fertile	Sterile	Percent sterile[2]	
Maryland, 1976						
1,600	55	2	1	1	50.0	1.8
200	76	3	1	2	66.7	1.3
Controls	49	32	31	1	3.2	63.3
Maryland, 1977 (dose response test)						
200	172	28	1	27	96.4	0.6
50	161	47	17	30	63.8	10.6
12.5	192	37	5	32	86.5	2.6
Controls	186	116	104	12	10.3	55.9
Maryland, 1977 (spatial distribution test)						
200 (divided)[3]	283	40	3	37	92.5	1.1
200 (all low)[3]	322	54	4	50	92.6	1.2
Controls[3]	324	190	177	19	9.7	54.6

[1]Only data from females for which both egg mass and sperm determination data are available are considered here.

[2]The 1977 data were entered for analysis of variance as percentages transformed by $\sqrt{\text{arcsine}}$. For the dose response test, treatment effect for percent sterile (sterility factor) was significant at $P < 0.003$; effects for percent fertile egg masses were significant at $P < 0.0001$. For the spatial distribution test, treatment effects for both phenomena were significant at $P < 0.0001$. The 1976 test was not analysed because of insufficient data.

[3]Combined data for females placed at 6 m and 1.5 m.

Table 6.4–14.—*Evaluation of the "sterility factor" as seen in spray test results based on percent nonembryonating egg masses obtained from inseminated females, 1976*

Location and treatment	Total egg masses from inseminated females	Number fertile	Number sterile	Percent sterile
Massachusetts				
1976 2% NCR	10	10	0	0
1975 2% NCR	20	20	0	0
1976 4.3% NCR	6	5	1	16.6
1976 10% NCR	19	14	5	26.3
Conrel	30	29	1	3.3
Stauffer	69	61	8	11.6
MGK	113	111	2	1.8
Controls	204	194	10	4.9
Maryland				
1976 2% NCR	1	1	0	0.0
1976 4.3% NCR	5	4	0	0.0
1976 10% NCR	7	4	3	42.9
1975 2% NCR	9	8	1	11.1
Stauffer	23	19	4	17.4
MGK	46	43	3	6.5
Controls	88	88	0	0.0

hold up well in rain. A more weather-resistant formulation was sought and obtained.

Results from the 1976 spray tests in Maryland and Massachusetts demonstrated that an improved NCR 2.2 percent formulation was superior to six other candidate formulations as a carrier for racemic disparlure for achieving mating disruption of feral sentinel gypsy moth females. The improved performance of the 1976 2.2 percent NCR over the 1975 2.2% NCR was largely due to smaller particle size (250 μ maximum versus 400 μ maximum) and to the addition of an improved sticker. Consistently greater mating disruption occured in the sparser populations of Maryland compared with Massachusetts, demonstrating that mating disruption is an inverse density-dependent phenomenon.

In order to study various parameters of the mating disruption concept under conditions of a season-long, zero-order release rate, a technique utilizing a grid of wicks was developed in 1976 that caused season-long mating disruption of gypsy moth sentinel females. In 1977, this technique was utilized in dose response and spatial distribution tests. A consistent dose response was seen for mating reduction due to release of racemic disparlure from grids of stationary, slow-release dispensers (dose controlled by the amount of wick at each grid spot) in the subdefoliating population level present in the Massachusetts test area but not in the sparser population in Maryland. Results from the spatial distribution test indicated that disparlure released at 1.5 m was just as effective as disparlure released both at 1.5 m and 6 m. There were no observed differences in mating of females placed at either level.

An unexpected "sterility factor," at present unexplained, played a prominent part in these tests. It was manifested by sterile egg masses being laid by inseminated females and was significantly more prevalent in treated than in control areas. When this was taken into account, percent effective mating falls from 11.3 to 5.2 percent in the treated areas, and from 34.9 to 27.2 percent in the control areas, for the 1975 test. There was some evidence for the "sterility factor" for all tests at both locations in 1976, although the effect was less pronounced than in 1975. The "sterility

factor" was very pronounced in Maryland in both 1977 tests. It was responsible for decreasing overall percent mating to 0.6–1.2percent (compared to 54.6–55.9 percent for controls) for the treatments using 200 wicks per hectare, while the percent mating, when this factor was not considered, was up to 14.3–17.6 percent (vs. 54.6–59.8 percent for controls) for this dose.

The level of effective control (percent mating reduction plus "sterility factor") has been impressive in all wick tests conducted to date in Maryland (on the "leading edge" of the advancing gypsy moth population) when a dose rate of 200 6.5-cm^2 Hercon® wicks per hectare was used. If results from larger tests planned for the summer of 1978 are equally impressive, it is hoped that this system can be registered with the Environmental Protection Agency for use as a mating suppressant. This assumes that the "sterility factor" can be demonstrated to be a natural phenomenon and not an artifact related to the use of laboratory-reared females which were used as an evaluation tool. This technology will be useful along the "leading edge" against low-level gypsy moth populations in suburban areas where aerial spraying is impractical, in isolated wood lots, and around high-risk areas such as campgrounds, truck stops, etc. It could also be employed against low-level outbreaks in areas far removed from the main northeastern population if the outbreak area is known to be of limited extent

The 1978 tests are designed to demonstrate mating disruption but not population reduction. The necessary techniques for measuring changes in low-level populations are now being developed and tested. Once these are available, it will be possible to conduct a definitive test to demonstrate reduction of natural gypsy moth populations along the "leading edge" by the use of disparlure.

The Use of Disparlure in Combination With Chemical or Biological Pesticides

E. Alan Cameron

The results of a series of tests in the early 1970's, in which broadcast applications of disparlure were

utilized, led most scientists to the conclusion that mating disruption would be effective, if at all, only where the population density of the gypsy moth was very low. Pesticides offer one means of drastically reducing population levels. These two factors led Beroza et al. (1974*b*) to suggest as a reasonable strategy the suppression of the gypsy moth population by application of a pesticide during the larval stage, then following this by applying microencapsulated disparlure to disrupt mating among those few survivors of the first treatment. They stressed the inverse density-dependent nature of the effect of a broadcast application of disparlure and predicted elimination of isolated populations of the insect. These authors proceeded to outline a 3-year test, to be conducted preferably on an island, to validate their proposal and looked even further into the future in proposing "... a moth-free barrier zone extending from the Chesapeake Bay to Lake Erie to contain the moth within the infested Northeast Corridor." Ultimately, they proposed to move the barrier northward to shrink the geographic size of the infested area.

During the 1974 field season, Beroza et al. (1975*a*), working in Massachusetts, applied Sevin 4-Oil® (carbaryl) to a 6-km² island, separated from the untreated mainland by 0.5–1.5 km of water, at a rate of 1.14 kg AI per hectare each application in two applications separated by 11 days while larval development was in progress. One-third of the island was later treated with a microencapsulated formulation of disparlure at 20 g lure per hectare, a second third received traps at a rate of 25 per hectare, and the remainder of the island was not treated further. Treatment effects were monitored using Johnson traps with Hercon® wicks, traps baited with virgin female moths, and untethered virgin females placed in the plots. Females from both field-collected and laboratory-reared pupae were used at different times during the monitoring period. Preseason and postseason egg-mass counts were also obtained.

Preseason egg-mass counts on the island ranged from 1,334 to 2,935 per hectare in the several test areas and were only 7.9 per hectare on the untreated adjacent island used as a control. The authors note

that collapsing populations in the test area and the excessive pesticide dose confounded interpretation of the results but that "... the results did support the general conclusion that disparlure can be used to reduce the mating success of low populations of the the gypsy moth" (Beroza et al. 1975*a*). The one clear conclusion that can be drawn is that two applications of carbaryl very effectively suppress a population of gypsy moths; to ascribe any credit for additional suppression to the supplemental treatment with disparlure must be tenuous on the basis of the data. These authors recognized the difficulties inherent in drawing definite conclusions in the absence of "... a satisfactory method of quantitative assessment of low-level populations...."

A similar series of studies was conducted in Pennsylvania in 1974 by Cameron and Mastro (1976). Sevin 4-Oil® was applied at 1.14 kg AI per hectare at the normal treatment time, that is, when the majority of the larvae were in the third instar, and about 304 of 405 ha treated with pesticide were sprayed with microencapsulated disparlure at 20 g per hectare just prior to adult emergence. Cameron and Mastro were unable to identify any differences in population reduction in the pesticide-treated area compared with the pesticide and disparlure treated area when using postseason egg-mass counts, percent reduction from preseason egg mass counts, or proportion of fertile to infertile egg masses. Only naturally occurring insects within the test plots were used for these assessments. Fewer moths were trapped in Johnson traps, baited with Hercon® wicks, in the plots treated with pesticide plus disparlure than in the plots with pesticide alone. This finding prompted questioning of the validity of using baited traps instead of females to monitor tests designed to evaluate mating disruption caused by disparlure.

Similar results were obtained in other tests conducted in central Pennsylvania in which Dylox 1.5-Oil® (trichlorfon) or Thuricide® (*Bacillus thuringiensis*) were used in place of Sevin® as the pesticide with which to suppress larval populations.

During 1974 and 1975, a number of field trials were conducted in central Michigan, the objectives of which were to develop eradication techniques for use

against low-level, isolated infestations, and to refine survey and treatment evaluation techniques. A number of areas were treated with Sevin 4-Oil® and/or microencapsulated disparlure, and populations were evaluated by placing female moths and disparlure-baited traps in the study area. Because the populations in which the tests were conducted were extremely sparse, the incidence of mating of female moths placed in untreated plots was very low, and few males were captured in disparlure-baited traps. Therefore, it was impossible to evaluate the effectiveness of the various treatments, and the testing was discontinued (Schwalbe 1978).

In recent years, tests of applications of a pesticide followed by broadcast applications of disparlure have not been conducted, largely because much key and basic information on the ability of disparlure to disrupt mating at low population densities in its own right is simply lacking. No tests have been conducted using the recently registered chemical pesticides such as Orthene® or Dimilin®, or the virus Gypchek. Prudence would dictate that such testing might await advances in our understanding of behavior and perhaps much more information on the ability of (+) disparlure of disrupt successful mating of gypsy moth adults under natural conditions.

Precopulatory Sexual Behavior of the Adult Gypsy Moth

Ring T. Cardé

Introduction

The adult male gypsy moth's search for the virgin female is mediated in part by the female's emission of an airborne attractant, similar to the communication system of most moths (Roelofs and Cardé 1977). Recent reviews of the role of the attractant pheromone in the mating behavior of the gypsy moth include those by Doane (1976), Richerson et al. (1976a, b) and Richerson (1977). Sufficient new information, particularly on the chiral nature of the attractant, is available to warrant a new critical appraisal and to initiate conceptualization of models of the sexual signaling system of this species. Ulti-

mately, successful utilization of synthetic attractants (or analogues) either as a sensitive lure for detection and sampling or as a disruptant of mating will depend upon a more complete understanding of natural mating behavior and of the effect of these chemicals on this process than has been achieved to date.

In contrast to the intense research effort accorded the development of a mating disruption system based upon the omnipresence of synthetic attractant, there is only fragmentary experimental and observational data on the gypsy moth's natural mating behavior. Doane (1968) reported that virgin females protrude their abdominal tip, thereby exposing the surface of the pheromone gland that lies beneath the intersegmental membrane immediately anterior to the ovipositor. While protrusion often appears rhythmic, the rate is variable within and among individuals, and often the gland is exposed without periodic retraction. This behavior, which in the gypsy moth is exhibited only by virgin females and which accompanies the emission of the pheromone, is termed "calling." Females are capable of attracting males and mating within 2 hours of pupal eclosion (Doane 1968, Forbush and Fernald 1896).

Collins and Potts (1932), as had Forbush and Fernald (1896), characterized the male's flight as a zigzag (hence the common French name of the gypsy moth, *le zigzag*), "usually against the wind carrying the scent." Doane (1968) described male flight direction as upwind "at slight angles" in an area harboring native females (and therefore some airborne attractant). At 30 m or less downwind of a calling female, flying males may turn directly upwind and proceed to the female (Doane 1968). This positive orientation in which the male flies directly up the plume appears to be due to the initiation of direct upwind flight behavior instead of flight crosswind or at an angle upwind. ("Plume" generally has been used synonymously with "aerial trail" in the pheromone literature. In this review, these terms refer to areas where the odor is above the threshold concentration required to elicit the appropriate behavioral reaction and not to the actual dispersion pattern of the pheromone.) Males evidently were aided in location of the female at close range by visual cues presented by

the female (Doane 1968) or perhaps her resting site, typically a vertical silhouette such as a tree. Although these observations supplied the essence of the male's orientation mechanisms, more definitive studies had to await the deciphering of the female's chemical message.

Bierl et al. (1970) characterized the natural attractant from combined data on two components extracted from the female. One component possessed intrinsic behavioral activity, eliciting movement of antennae and free legs in males restrained by holding their wings together over their thorax. The second component, an olefin, became behaviorally active when treated with m-chloroperbenzoic acid to yield an epoxide. The identified compound, cis-7,8-epoxy-2-methyloctadecane, was synthesized as the racemate. In field trials, Beroza et al. (1971b) reported that synthetic ($\pm$) disparlure at dispenser doses of 1-6 μg was equivalent to the attraction power of a laboratory-reared virgin female. (The ($\pm$) disparlures employed in these experiments contained from about 5 to 15 percent racemic trans isomer. However, in only a few cases was the percent trans contamination stated. The ($\pm$) disparlures are identified according to the senior author of the paper describing the synthetic procedure.) These field experiments were conducted in advance of the natural flight and used released, laboratory-reared males. Of the males released, 15-16 percent were captured by the treatments baited with ($\pm$) disparlure; catches in the traps baited with females were comparable to those elicited by the synthetic attractant. These data suggested that the synthetic attractant and the natural pheromone were identical, and so emphasis was directed toward utilization of this compound in management programs rather than a continued study of the pheromone chemistry.

However, the epoxide moiety of disparlure is chiral, so that (+) and (-) enantiomers exist. Several synthetic routes for the stereospecific syntheses of (+) and (-) disparlure have been developed (Farnum et al. 1977, Iwaki et al. 1974, Mori et al. 1976), and field tests confirm that disparlure containing 94 to 99 percent (+) enantiomer is roughly fivefold to tenfold more attractive than the racemate (Cardé et al. 1977b, 1978, Miller et al. 1977, Plimmer et al. 1977a). No method

is yet available to assess the absolute or relative enantiomeric purities of the disparlures synthesized by the three reported stereoselective syntheses (Farnum et al. 1977, Iwaki et al. 1974, Mori et al. 1976). The optical purities of the compounds used in these studies have been assumed to be identical with the estimated composition of synthetic intermediates, although racemization in the conversion to the epoxide cannot be excluded until a technique for the enantiomeric analyses of disparlure is developed. The natural gypsy moth pheromone is likely similar to the (+) disparlures evaluated, but rigorous chemical confirmation of the chirality of the natural attractant awaits development of a suitable analytical technique applicable at the microscale level. When emitted with the synthetic (+) enantiomer in the field, the (-) enantiomer acts as an antagonist, decreasing the likelihoods of upwind orientation and landing near the chemical source (Cardé et al. 1977b). Most of the behavioral observations conducted in the past have evaluated ($\pm$) disparlure as the stimulus, so that most published interpretations of the male's behavior must be viewed cautiously.

A comprehensive model of the sexual communication system of this species would detail the sequences of behavior that occur in mate location and copulation; quantify the roles of olfactory, visual, and tactile cues; and delineate the atmospheric dispersion pattern of the chemical signals. The utility of such a model would be manyfold. For example, interpretation of catches of attractant traps used for survey and detection is problematic in that the relationship of the numbers trapped to the actual population levels is unknown. A model would describe the adult male's flight pattern in the absence of the pheromone, the active space generated by the lure and the likelihood of capture for males that enter the active space. (Active space is usually defined as the area containing at least the minimum atmospheric concentration of pheromone required to elicit a particular behavior. A dynamic concept of the active space would include maximum concentration, individual variability in thresholds, and, particularly, the repsonse latencies (Cardé and Hagaman 1979, Hagaman and Cardé 1978). The likelihood of response is highly dependent

upon the time spent in a given pheromone concentration.) Then, for a given trap density the trap catch might be related to absolute male density.

Similarly, in some population levels the process of mate location evidently involves a switch in behavior from crosswind (or random) flight direction to direct upwind flight to the lure. The cue initiating the change in behavioral state could be the rate of change in atmospheric concentration of attractant when the moth enters a plume. Knowledge of these rates and of the active space generated by a calling female could suggest the atmospheric concentration of synthetic attractant required to camouflage the changes in concentration at the edge of a natural plume. This prediction could suggest specific atmospheric concentrations of disruptant to be evaluated instead of the current practice of empirical screening of one or several doses for disruptive effect.

Conceptualization of the mechanisms regulating the male's location of the female has numerous pitfalls. Models may tend to deemphasize the variability of behavioral responses, regardless of the causative nature of the variation (genetic, age dependency, diel cycle dependency, previous behavioral experiences, nutrition, pathogen infection, etc.). And sequence analysis may tend to emphasize first-order interactions between various behaviors, for these relationships are the most easily studied and obvious. Notwithstanding such difficulties, models of gypsy moth behavior provide foci for defining testable hypotheses. Because the precise relationships between the behaviors and control systems have yet to be verified, most of the following hypothetical models are presented with open-loop control systems (lacking feedback parameters to adjust the previous input) and without return loops to earlier behaviors, although feedback control adjustments and return looping of behaviors undoubtedly occur.

Female Behavior

Fernald (Forbush and Fernald 1896) noted that the female, after eclosion, crawls a "short distance" from the pupal case (recent observations reveal that this distance is often less than 5 cm) and expands her wings. Protrusion of her ovipositor (calling) begins shortly after (seldom before) complete wing expansion. The calling behavior usually is accompanied by a slight elevation of the wings, an arrangement that likely facilitates the male's lateral approach. Males that arrive at the females are in a behavorial state of walking on the substrate while wing fanning. Generally they approach the female laterally with tarsal contact on the side of her body or wings. Males then curve their abdomens toward the tip of the female's abdomen, engage their claspers and drop to an end-to-end position. Copulation lasts at least 30 minutes; a mean copulation time of about 1 hour appears normal (Doane 1968, Fernald in Forbush and Fernald 1896). Transfer of suffient sperm to fertilize at least 95 percent of the ova of all females tested occurs within 8 minutes. At the termination of mating, the female shakes off the male. She often disperses from the mating site (particularly if the location is in full sunlight) by walking downward and toward the shade (negative photo and/or positive geotaxis) (Doane 1968).

In many moth species, the female may accept or reject a courting male by her behavior. In some such species, a possible sexual selection process might be involved when the male disseminates a courtship pheromone and causes the female to choose him over other males (Baker and Cardé 1979). But in the gypsy moth there is as yet no evidence either that receptive females reject any of the males attempting copulation or that males produce a courtship pheromone.

Calling behavior and emission of pheromone do not occur continuously in most moth species; rather, these events occur nearly simultaneously and only during discrete daily intervals. In the moth species that have been investigated, the periodicity has a circadian (endogenous) basis, and often temperature shifts the exact calling time within a "gate" (Cardé et al. 1975a). The circadian basis and possible temperature interactions with calling and emission have not been studied in the gypsy moth, but Richerson and Cameron (1974) have shown that the rate of pheromone emission does possess a diel cycle. Females evidently emitted disparlure continuously, albeit at low levels, and the maximum emission rate of females (collected as pupae in the field) occurred

between 1000 and 1530 hours, when a female could emit as much as about 800 ng in a 30-minute interval. Interestingly, laboratory-reared females exhibited a rather low and constant rate of disparlure emission (about 5 ng per 30 minutes) with no discernible diel periodicity.

Evidence from the field also supports a diel cycle of emission. Cardé et al. (1974) found that maximum catch of males in traps baited with females or synthetic (±) disparlure occurred between 1100 and 1500 hours. The catches of males in (±) disparlure-baited traps and in female-baited traps were coincident: Males were not caught in synthetic-baited traps (which emit attractant continuously) unless they were also being caught in female-baited traps. These data suggested that the key factor influencing the attraction rhythm was the diel response of the male rather than a rhythm of calling by the female (Cardé et al. 1974). This pattern of regulation of attraction periodicity may be general within *Lymantria* and perhaps other Lymantriidae, for Schröter and Lange (1975) noted in *Lymantria monacha* L. a synchronous attraction of males to calling females and synthetic lure ((±) disparlure). In the gypsy moth, the female's rhythm of rate of emission (Richerson and Cameron 1974) would influence the active space of pheromone that she generates and hence her relative "attractiveness" when contrasted to a synthetic lure that emits attractant at a relatively constant rate. A daily change in the relative trap catch elicited by females and synthetic lure was noted by Cardé et al. (1974): During mid-afternoon the females were more attractive than (±) disparlure baits, while during the early evening this pattern was reversed.

The female's pattern of behavior appears uncomplicated. She evidently exercises no selection in the choice of a particular male suitor for a mate. Her calling rhythm seems relatively inconsequential in the determination of the time of attraction of the male. Of considerable interest, however, is her variable rhythm of emission. This variability in rate of release of attractant suggests that the active space of pheromone that she generates varies considerably during the day. The precise amounts of pheromone released (including variation among individuals and the effects of virus infection and nutrition) are critical parameters

to quantification of communication distance in this species.

Male Behavior

Appetitive Behavior

In the previous studies of the sexual behavior of the gypsy moth, the mechanisms, in particular the olfactory cues that the male moth uses to locate the female, understandably have been given much attention. However, it is important to distinguish between pheromone- and nonpheromone-mediated preconsummatory behaviors. The male gypsy moth flies in absence of the pheromone and assuredly it is this appetitive, preconsummatory flight that determines much of male dispersal, particularly in sparse populations where background pheromone concentrations are probably below the threshold for eliciting any higher level male behaviors. In this classification of behavioral states, patterned after Craig (1918), consummatory acts would include all behavioral states elicited by the female's pheromone (and accompanying cues) from the initiation of positive orientation by the male until termination of mating. Appetitive behavior in the gypsy moth would be flight preceding the consummatory behaviors and could be viewed as the "searching phase."

It can be presumed that the most advantageous appetitive flight behavior in "search" of a pheromone source would be to fly crosswind. This average vector would be most apt to traverse a pheromone plume and simultaneously exercise the least energetic expenditure by the male. Flight upwind, in absence of a pheromone, would prove the most costly in terms of energy expended versus the probability of finding a virgin female. Few observational data on purely appetitive flight exist, for most observations of male behavior have been conducted in areas with at least moderate populations of adults. If the average vector of appetitive flight direction tends to be crosswind, then this could be termed "crosswind anemomenotaxis" (Kennedy 1977). (Anemomenotaxis is directed orientation that employs wind as a compass.)

Investigation of appetitive, preconsummatory flight (as opposed to migratory flight of some moths) is a parameter of consequence to interpretation of

attractant-baited trap catch in peripheral, sparse infestations. Surprisingly, there is practically no information on this behavior. Kannowski and Johnson (1969) observed that "patrolling" *Formica* (Hymenoptera: Formicidae) males (evidently in appetitive flight) usually fly crosswind prior to attraction to females, and Steiner (1953) observed *Geotrupes stercorarius* L. (Coleoptera: Geotrupidae) in "strong" wind flying "perpendicular" to the wind axis prior to upwind flight along odor trails of cow-dung pats. These observations are suggestive of a strategy common to appetitive flight prior to olfactory, inflight attraction, but confirmation of this pattern and the requisite wind field will require quantitative behavioral and meteorological appraisal.

Communication Distance and Active Space

Some of the early studies with the gypsy moth involved the release of males at varying distances "downwind" of females in an effort to ascertain the apparent communication distance. Fernald (Forbush and Fernald 1896) reported that a few males could be recaptured when the distance between the male release site and the females was 800 m. Collins and Potts (1932) in more extensive trials liberated marked males leeward of females and also reported "attraction" of wild males to females on coastal islands, a test protocol that eliminated unknown intervening pheromone sources.

In all of these trials the implicit assumptions of interpretation include: Arrival of a male at a female indicates positive orientation over the entire distance, and wind fetch is unwavering and directly toward the males from the females. In reality, an unknown proportion of the distance travelled could be under anemomenotactic guidance in the absence of pheromone or even due to random flight direction, and thus would not be a positive anemotactic reaction to the attractant.

Nonetheless, Bossert and Wilson (1963, Wilson and Bossert 1963) have used Collins and Potts' data to conclude "that a group of 10–15 females together have a maximum 'reach' of between 3.72 and 16.09 km,

[an] estimate in full accord with the results of numerous field studies made on other moth species." The lower value is based on the catch of four gypsy moth males at a trap baited with 12–15 females on an offshore island, and the larger estimate "being about the extreme limit of sex attraction in the Lepidoptera." Using estimates of the Q/K (or ratio of the number of molecules released to the threshold density) per female and the likely "persistent sea breeze ... about 450 cm/sec or more," Bossert and Wilson (1963) estimated the downwind communication distances of a single female gypsy moth to be 1,820 m at a windspeed of 500 cm per second to as much as 4,560 m at 100 cm per second. These conclusions are doubtful.

Aylor et al. (1976) have estimated, from direct wind field measurements in the forest environment and relative turbulence theory, that the average pheromone concentration within several meters of a pheromone source may be as much as 25 times lower than peak instantaneous concentrations. This disparity would be caused by a meandering of the plume in a turbulent wind field, as was pointed out by Wright (1958). Validation of importance of peak vs. average concentrations was attempted by observing caged males 1.2, 2.5, and 5 m downwind of a known emission rate of (±) disparlure and determining the proportion of wing fanning response (Aylor et al. 1976).

In a wind tunnel bioassay when the dosage of (+) disparlure is held constant, the (+) disparlure stimulus does appear to evoke wing-fanning behavior with about the same latency and proportion as a (±) disparlure stimulus (Cardé and Hagaman 1979, Miller and Roelofs 1978). However, in a wind tunnel assay, decreasing concentrations of (+) disparlure require longer stimulus presentation periods until the wing-fanning response occurs (Cardé and Hagaman 1979), so that male response may be dependent on duration of the stimulus as well as either instantaneous or average concentrations. Aylor et al. (1976) did not report latencies to wing fanning, and their studies have not been integrated into an estimate of active space. They emphasized that, at least close to

the source, average concentration may not fully explain the male's behavioral reactions.

Olfactory Modulation of Upwind Orientation

Kennedy (1977) has reviewed the evidence for various mechanisms of orientation to distant odor sources. He argues cogently that in flying organisms the most plausible mechanism for distance attraction to a chemical is an odor-induced, positive anemotaxis. This acts, at least close to the odor source, in concert with an orthokinetic, optomotor modulation of groundspeed. (Orthokinesis is a change in the linear velocity of locomotion and it is dependent on the stimulus intensity.) In the gypsy moth, the female's pheromone would initiate male flight upwind (positive anemotaxis), which necessarily requires a visual appraisal of progress (optomotor feedback). Males flying upwind usually zigzag along the plume path, making excursions to the right and left from the wind vector. This reversing anemomenotaxis is evidently initiated when the male reaches the "plume" margin with its attendant decrease in pheromone concentration. According to Kennedy, males should not exhibit reversing anemomenotaxis if the wind field is uniformly permeated.

The available evidence on orientation maneuvers of the gypsy moth generally comes from two kinds of information: Field observations where the structure of the active space and wind field must be inferred, and wind tunnel observations where the plume and visual parameters can be controlled to a great extent. Unfortunately, the latter situation creates an idealized aerial trail that may not accurately reflect the filamentous and perhaps disjunct chemical plume generated in the forest habitat of this species (Aylor et al. 1976, Wright 1958).

In observations of male flight in an area with wild females and thus presumably with some natural background of pheromone, males were reported to fly at "slight angles to the wind" (Doane 1968) without a reversing anemomenotaxis. This observation suggests that male flight in the presence of low atmospheric concentrations of attractant could be an anemomeno-

taxis angled upwind, although it is also possible that this flight vector is not modulated by pheromone and hence is actually appetitive flight. Obviously more comparative studies of male flight behavior in wind field will be necessary to resolve this point.

Reversing anemomenotaxis could be initiated when the in-flight male encounters a steep enough change in attractant concentration (or perhaps a threshold value) to elevate the peripheral sensory receptor firing rate discernibly above the background firing rate. Integration of the signal to noise ratio can occur over comparatively long intervals. Response latency at low (+) disparlure stimulus levels is several minutes (Hagaman and Cardé 1979). Males then can initiate upwind anemotaxis, employing reversing anemomenotaxis whenever their flight path carries them to the plume edge.

The factors initiating the change from either anemomenotactic crosswind or random appetitive flight to positive anemotaxis (or perhaps an anemomenotaxis angled upwind in low concentrations of pheromone) are likely to be difficult though not intractable to determine. The rate of change in concentration at the edge of the plume probably constitutes the most important factor in determination of an active space where the olfactory cue is a single compound (as is thought to be the case for the gypsy moth) or in multiple compound systems where the ratio of components is not particularly critical to the behavioral response (Cardé et al. 1977a). Two factors will increase the discernibility of the plume boundary: An increase in the steepness of the gradient or a decrease in the speed of flight.

Theoretically, a male that has flown into an active space should be able to follow the path directly to the female using the mechanisms outlined by Kennedy (1977). In practice, however, males may be unable in moderate-to-dense populations to use reversing anemomenotaxis to fly to females because of the lack of discernible plume boundaries. This hypothetical effect would result from the multitude of natural emitters and perhaps the roughness of the forest canopy generating a mechanical turbulence (see, for example, Raynor 1971) that mixes the plumes,

thereby destroying sharp plume boundaries. In such a situation, upwind anemotaxis coupled with reversing anemomenotaxis might be an ineffective strategy for efficient mate location, and if so, it is apparent that additional reactions must supervene.

Klinokinesis is the frequency (per unit time) of random (nondirected) turning being dependent upon the stimulus intensity. A klinokinetic flight, therefore, would be characterized by an increase in the rate of random flight turns in response to an increasing gradient, and it is probable that females of a geometrid moth, *Cidaria albulata* L., use this mechanism in flight location of the oviposition host (Douwes 1968). In the field, klinokinetic flight could be coordinated with visual cues (the evidence for odor-conditioned visual orientation has been summarized by Kennedy 1977). It is doubtful that the male gypsy moth employs a strictly klinokinetic reaction to locate the female, although this mechanism hypothetically could be of importance in some situations—for example, relatively still air with a sufficiently steep odor gradient. On the other hand, evident odor-modulated orientation to vertical silhouettes has been observed with this species. Richerson et al. (1976b) reported that males in a dense natural population "... did not orient directly, either in a zigzag flight or in a straight line oriented run [flight] over long distances to calling virgin females. All males oriented to vertical silhouettes, such as trees, stumps, shrubs and upright boulders whether females were present or not. ... Search [flight] up and down appeared to be random." Cardé et al. (1975b) also noted vertical flight near trees (flight up and down, usually within 1 m of a tree) in 25 percent of the males entering 0.01-ha plots within which 110 mg per hectare per day of synthetic (±) disparlure was emitted. Trees also may modify plume characteristics, complicating any interpretation of the effects of visual cues.

Richerson's (1977) observations that no evident anemotaxis (or reversing anemomenotaxis) occurs when numerous natural sources of pheromone are present has led him to conclude that in dense populations "pheromone-stimulated males" use "a number of different stimuli (chemical, visual, and tactile) in order to initiate short-range behavior and to locate and be stimulated to attempt to mate with the test females."

Although quantitative data on the atmospheric concentration of pheromone and its dispersion in the wind field in various population densities are not yet available, it appears that, compared to mating in sparse adult densities, the male's location of the female differs considerably in high population density. Among the factors initiating this change could be either a specific absolute atmospheric concentration of pheromone or the lack of steep pheromone gradients, including specific meteorological conditions such as low wind velocity, turbulence, and vertical mixing. There is no evidence that vertical flight behavior near trees is regulated by a klinokinetic mechanism.

For males that successfully locate females, the vertical flight state may be followed by a change to stationary hovering, reversing anemomenotaxis, or landing. The precise situation evoking the change from vertical flight to, for example, the reversing anemomenotactic response, could be a change from the evenly permeated atmosphere into a discernibly steeper gradient of pheromone or a threshold boundary. A possible chemotactic orientation would require a discernible concentration gradient, a cue that could exist only very close (for example, <1 m) to the female emitter. However, chemotaxis remains in flying organisms unproven as an orientation mechanism. In contrast, in Lepidoptera with multicomponent pheromone systems where the ethological function of the components is known, close-range behaviors (including landing) can be mediated in part by secondary components (Roelofs and Cardé 1977).

Returning to male behavior in sparse populations, a different sequence of behavior may intervene between the states of reversing anemomenotactic flight and landing. As the moth nears (within decimeters) the chemical emitter, the atmospheric concentration of pheromone may be raised perceptibly, and visual cues such as a tree trunk may modulate the moth's airspeed via an optomotor orthokinesis. These factors also could induce landing.

Behavior After Landing

Landing signifies tarsal contact with the substrate, and males in this behavioral state wing fan while walking (wing fanning while stationary can occur intermittently for short intervals in some individuals) until location of the female or return to an earlier behavioral state. The cues promoting successful location of a female after landing again may be dependent upon the background concentration of pheromone. In low population densities, some males have been observed to fly directly either to the female or to within a few centimeters (Doane 1968, Doane and Cardé 1973).

Males on a vertical substrate in the wing-fanning/ walking state generally maintain a body orientation relatively perpendicular to the ground and move on a tree trunk laterally, horizontally, or obliquely, often with reversals and changes in direction. Wing fanning probably draws air over the antennae and may aid the male's sampling of airborne chemical cues. Speed of walking appears to remain relatively constant. This pattern has not been quantified, so several orientation mechanisms remain plausible. As noted by Kennedy (1977), klinokinesis and klinotaxis or tropotaxis are possible reactions of walking insects to olfactory attractants. (Positive tropotaxis is direct orientation by turning toward the most stimulated side; this mechanism requires a paired set of intensity receptors.) Formal demonstration of the mechanisms of walking orientation of the gypsy moth male at the decimeter range will require new and definitive tests. Thus the strategy and mechanisms of male search patterns after landing has been induced remain a critical behavior to be elucidated.

Role of Vision in Orientation and Recognition of the Female

Doane (1968) noted that males would alight on a cage containing a mated (nonattractive) female on a 0.3-m stake when the cage was 0.15 m downwind of a virgin female and therefore in her attractant plume. Although this test suggested that vision in the presence of the sex attractant was important to

landing, it did not establish the precise visual cues eliciting landing (for example, the stimuli presented by the female or her resting site).

Richerson (1977) investigated the role of visual cues presented by the female through the presentation of live females as well as females modified by rinsing in solvent, abdominal scale or wing removal, or painting the females red, white, or blue. He found that mean mating attempts were highest for unmodified and wingless females. But he also found that females, regardless of how they had been treated, elicited mating attempts; even solvent-washed females evoked more than one-tenth as many mating attempts as untreated virgin females. Further, the presence of a dispenser of (±) disparlure adjacent to the females neither elevated nor suppressed the response level. Because (±) disparlure is a combination of both an attractant and an antagonist, the apparent lack of influence of (±) disparlure in this experiment is ambiguous. Richerson (1977) concluded that "males stimulated by the pheromone utilized a number of additional stimuli to initiate and terminate sexual behavior patterns," but the specific role of visual cues is not evident in this test.

As noted earlier, visual cues such as the calling site could induce orientation, either directly to the female's location or via vertical search flight. This was investigated (table 6.4–15) by placing synthetic (+) disparlure sources either on tree trunks of 0.3 or 0.07 m in diameter, or suspending the dispensers by a black string 3 m away from any trees. Analyses of the behaviors show that more males flew to within 2 m of the synthetic dispensers on the 0.3 m trees than to the dispensers that were on saplings or suspended. Of those moths that flew to within 2 m of dispensers, the proportion that landed and wing fanned on the cage was higher for the males flying to the 0.3 m trees. These observations suggest that, in the presence of synthetic (+) disparlure, visual cues of the typical resting site modulate the male's close approach and landing. A complete separation of visual cues from changes in wind field and possibly the plume modification caused by trees will be difficult to achieve experimentally.

Table 6.4-15.—*Effect of the diameter of a vertical silhouette upon male gypsy moth in-flight orientation within 2 m of a 10-μg (+) disparlure source on a cotton wick (Iwaki et al. 1974) and landing on the chemical source or silhouette[1]*

Wick placement	Number of males observed within 2 m of source[2]	Number of males wing fanning on source[2]	$\bar{x}$ sec (±SD) wing fanning on source (fanners only)[3]	$\bar{x}$ sec (±SD) fanning on trunk or source (fanners only)[3]	$\bar{x}$ sec (±SD) orientation flight within 2 m of source (all males)[3]
0.3 m tree trunk	65a	37a	28.1a±42.2	41.6a±67.0	62.1a±78.5
0.07 m tree trunk	38b	12b	16.9a± 7.0	29.4a±45.5	34.6b±41.5
Suspended in cage	34b	5b	28.8a±40.7	28.8a±40.7	30.5b±30.8

[1]Test conducted on July 19, 1976, in Eastford, Conn. All dispensers were held in a 3-cm-diameter X 5-cm-long screen cage and placed on either oak trees or saplings at a height of 1.8 m. The suspended cage was held 3 m from any saplings or trees by a string. Observers and treatments were rotated to new sites every 15 minutes during the 2 hours of observation. Equal observation time was accorded each treatment. No calling females were evident within 30 m of the test site.

[2]Numbers in the same column not having the same letter are significantly different from the null hypothesis according to χ^2 (P<0.01).

[3]Means in same column not having the same letter are significantly different according to the *t*-test (P<0.05).

A similar experiment (table 6.4-16) was conducted using white cotton (+) disparlure dispensers about the size of a female and dead females that had been rinsed several times with acetone. Treatments were placed in open and concealed (artificial bark flap) sites. There was no evident difference in mean time between arrival within 2 m of the treatment and direct contact with the (+) disparlure dispenser, suggesting that visual cues presented at close range by the female are not very critical to the male's location of the female. Indeed, a dead, acetone-rinsed female alone evoked no male reactions.

It is evident that most theoretical mechanisms proposed for the male's location of the female in the field remain to be proven rigorously. The current view of the sequence of male behaviors and the stimulus variables regulating these reactions in the field is summarized in figure 6.4-5.

Table 6.4-16.—*Role of visual cues presented by a female in speed of male location of (+) disparlure (Iwaki et al. 1974) sources[1]*

Treatment	$\bar{x}$ time (sec) ±SD from initial orientation (<2 m) until direct contact with treatment[2]	Number of observations
10 μg (+) disparlure on cotton wick concealed under bark flap	43.5±51.9	13
10 μg (+) disparlure under acetone-rinsed dead female	49.2±52.7	27
Acetone-rinsed dead female	No orientations	0

[1]Treatments were placed >15 m apart at a height of 1.5 m on tree trunks (*Quercus* (oak) spp. or *Carya ovata* (shagbark hickory)) of 0.3 to 0.5 m diameter. Three observers rotated among treatments at 15 minute intervals, at which time treatments were moved to new trees more than 15 m away. Males not locating treatments are not tabulated in these data. Test conducted from 1330 to 1530 hours e.d.s.t. in Eastford, Conn., on July 20, 1976.

[2]Includes all male behavioral states (flight, landing, walking, wing fanning) until direct contact with treatment (tarsal or wing).

Diel Periodicity of Sexual Response in the Field

The daily rhythmicity of the male's attraction to synthetic (±) disparlure and virgin females was investigated by Cardé et al. (1974). The main period of activity commenced around 0900 and terminated around 2100 hours. Peak numbers were lured between 1100 and 1500 hours, and a second period of activity (predominantly to synthetic attractant) evidently modulated by temperature sometimes occurred between 1900 and 2100 hours.

Wind Tunnel Studies of Response to Disparlure

The controlled milieu of the laboratory allows study of several behavioral states that cannot be
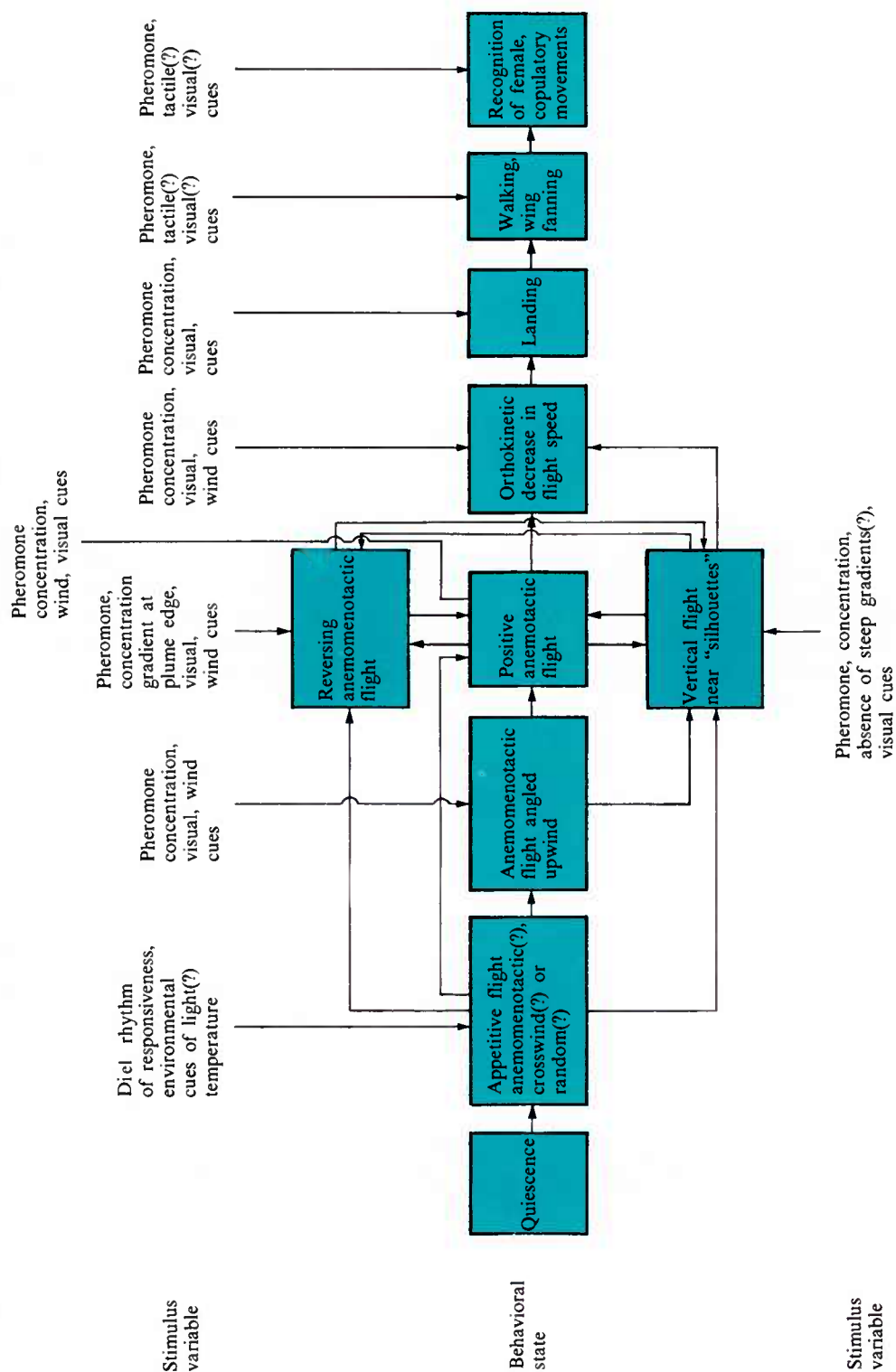
Figure 6.4-5.— *Model of possible reactions of a wild gypsy moth male to a virgin calling female. Loops for behavior returning to previous states are excluded.*

readily observed in the field and rigorous control of plume characteristics of the test chemical. Miller and Roelofs (1978) and Cardé and Hagaman (1979) have studied several aspects of male gypsy moth response to enantiomers of disparlure in wind tunnels that provide an optomotor feedback to the in-flight male by unrolling underneath the tunnel a striped pattern (figure 6.4–6), a bioassay system first employed with moths by Kennedy and Marsh (1974). Such wind tunnels allow the experimenter to regulate the male's upwind progress. By coordination of the optical feedback of the floor movement with the individual male's flight speed, the male can be induced to fly in an attractant plume in the center of the wind tunnel plume for up to several hours. The bioassay system allows a number of preflight behaviors and the in-flight orientation responses to be quantified by direct observation or recorded on videotape for subsequent analysis. This technique has been used for the male gypsy moth by Cardé and Hagaman (1979) and Miller and Roelofs (1978) to determine the effects of the (−) enantiomer on responses to the (+) disparlure; by Cardé and Hagaman (1979) to establish dose response relationships for (+) disparlure; and by Hagaman and Cardé (1978) to document the sequencing of preflight behaviors.

A quiescent male could respond to the presence of pheromone by antennal movement, body movement, leg movement, walking, slight wing movement, wing fanning, wing fanning concurrent with walking, flight, and copulatory attempts. In numerous studies of the pheromone-mediated behaviors of moths, orderings, sequences, and hierarchies of stimulus-response relationships have been assumed, usually without precise definition of terms or direct proof. Schwinck (1958) emphasized the evident inverse relationship between stimulus intensity and response latency (reaction time) in *Bombyx mori* L. (Lepidoptera: Bombycidae). Cardé and Hagaman (1979) and Hagaman and Cardé (1978) confirmed this relationship in a series of dose response studies with the male gypsy moth. The earliest components of sexual behavior (antennal movement, body movement, leg movement, and slight wing movement) were most frequently observed in response to low concentrations of pheromone. However, these early components were almost always followed by wing fanning within one second, indicating that, even at low-stimulus intensities, the probabilities of eliciting all of these behaviors are essentially equal.

The behaviors of wing fanning and walking while wing fanning are almost always followed by flight. In
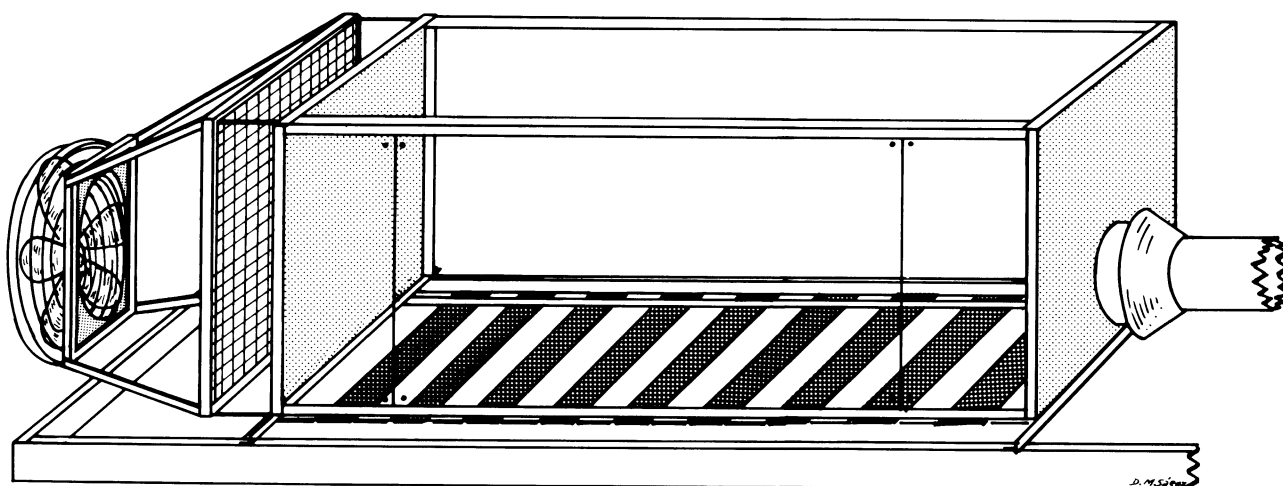
Figure 6.4–6.—*Wind tunnel for bioassay of attractant pheromones. Air flows from left to right.*

the wind tunnel where the aerial plume dimensions can be specified, gypsy moth males engage in upwind optomotor anemotaxis in concert with reversing anemomenotaxis. When the test stimulus was 100 ng of (+) disparlure, sustained upwind flight could be induced in 50 to 100 percent of the males tested (Cardé and Hagaman 1979, Miller and Roelofs 1978). The highest doses tested (1,000 ng) evoked a lower average ground speed of flight (the amount of floor movement necessary to maintain stationary flight in the plume), indicating that an optomotor, orthokinetic modulation of ground speed could be involved in orientation to a female at close range.

Another interesting finding was the variation in mean speed of flight within given stimulus levels. For example, at the 1,000 ng stimulus level, mean ground speed ranged from 0 to 6.6 m per minute, while the mean of all individuals was 2.6 m per minute. This variation may relate to optimum strategies for mate location in different wind fields. Where the plume is well defined (little wind turbulence), a comparatively rapid flight speed might be successful in plume following; in a turbulent wind field where the plume meanders, a relatively slow flight speed may be most successful in maintaining flight within the plume (Cardé and Hagaman 1979).

In the field, the (-) enantiomer of disparlure acts as an antagonist to the attractiveness of (+) disparlure. In the wind tunnel, the addition of (-) enantiomer to (+) disparlure (particularly when they are admixed in a 1:1 ratio for a nominate (±) disparlure) decreases the time spent in positive anemotaxis, the orthokinetic speed, and, therefore, the distance travelled. The likelihood of preflight wing fanning and its latency was unaffected by the addition of (-) enantiomer. The sequence of these reactions and the stimulus variables are summarized in figure 6.4-7. Obviously, the behavioral repertoire is highly truncated compared to the male reaction in the field.

Antipredator and Competition Effects on Behavior

Doane and Cardé (1973) observed that when two or more males came into wing-tip contact while in either the hovering or the wing fanning/walking behavioral states near a caged, virgin female, the males often flew away within 1 or 2 seconds. Termination of close-range behaviors could be interpreted as sexual competition between males or as an antipredator escape mechanism that supervenes sexual behavior. Richerson et al. (1976b) reported that male wing touching resulted in a "spacing out of males," but not termination of searching by flight away from the encounter site.

Ultrasound elicits in pheromone-orientating males evasive flight maneuvers in both field and wind-tunnel flight (Baker and Cardé 1978). This behavior probably originally evolved in response to predation by bats, which emit ultrasound to locate flying prey, and has been maintained by the selective pressure of bat predation that could occur during dusk and early evening moth flight. Interestingly, the evasive reactions of the moths can be evoked during daylight flight in the field and during pheromone-mediated positive anemotaxis both in the field and in a wind tunnel, indicating that this presumed antipredator behavior can take precedence over sexual behavior. Because significant ultrasound can be generated by laboratory equipment or even breaking of twigs in the field, knowledge of this response is of practical concern to studies of sexual behavior.

Trap Catch Elicited by (+) Disparlure and by Disparlure Analogues and Combinations

In the study of pheromones, the presence of a positive trap catch has been considered demonstration of "attraction." As Kennedy (1972) correctly pointed out, the actual behavior involved could be something quite different, such as inducement of landing. Kennedy has suggested that in many of the so-called behavioral investigations of the specificity of attraction, it is not so much the behavior of insects that is being studied but the "behavior" of chemicals. Thus attractants have synergists, inhibitors, and masking agents, all of which imply a mode of behavioral effect that remains to be established. Counting males caught in a trap does not constitute a study of behavior. And, for trap catch to have any
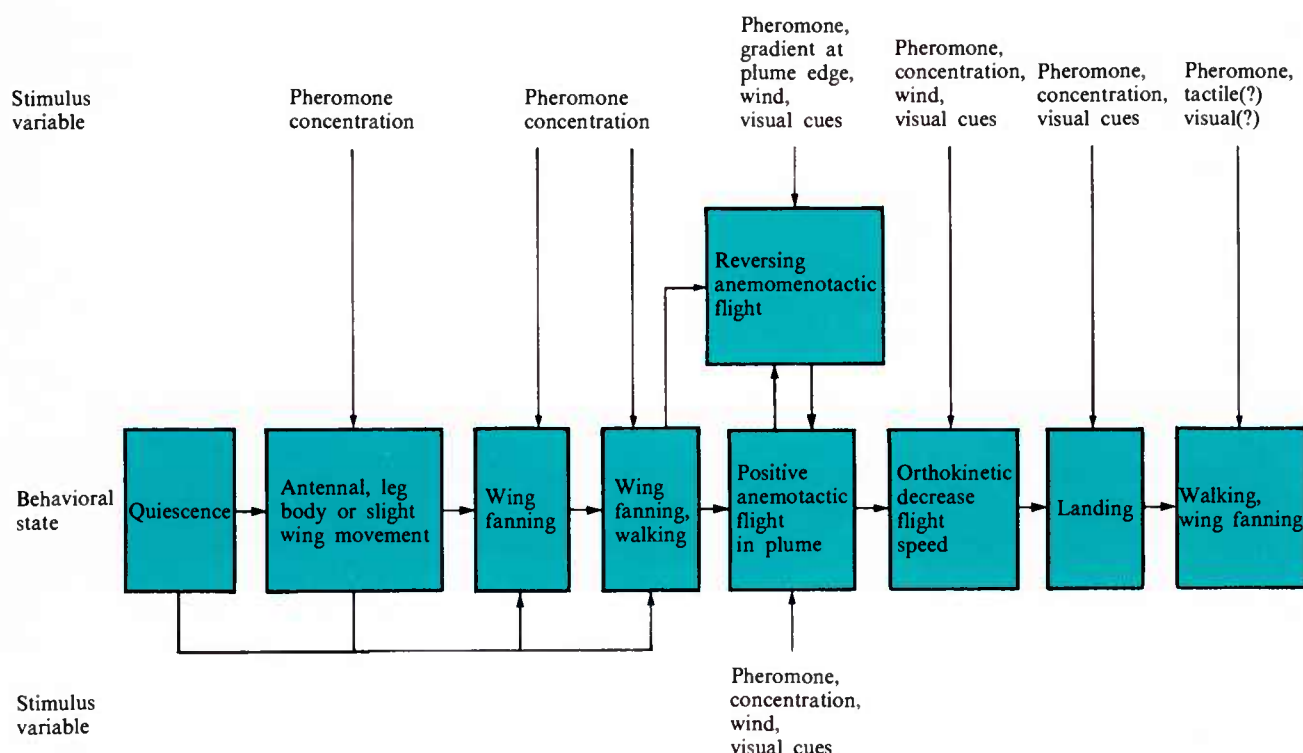
Figure 6.4-7.—*Model of male gypsy moth behavioral reaction to airborne synthetic attractant in a laboratory wind tunnel. Loops for behaviors returning to previous states are excluded. The moth is presumed to be responsive.*

significance, baited traps must be compared to unbaited traps by appropriate statistical protocols. This criterion has not been met in many of the studies with the gypsy moth. Carle (1975, 1976) claimed that many of the terpenoid compounds identified as pheromone components of scolytid beetles also "attract" the gypsy moth. Similarly, Sheads et al. (1975) also reported "attractancy" of numerous compounds structurally analogous to disparlure, except that the epoxide was replaced by a number of other moieties. Cardé et al. (1977c) reinvestigated the trap catch elicited by some of the compounds tested by Sheads et al. and noted that the disparlure analogues elicited trap catches statistically indistinguishable from unbaited, control traps. Schneider et al. (1974) also evaluated racemic epoxy analogues of

(±) disparlure; these compounds apparently did lure males but clearly were less effective as lures than (±) disparlure.

Recently available (+) disparlures are roughly fivefold to tenfold more effective as trap baits than (±) disparlure (Cardé et al. 1978), and the (+) enantiomer elicits an increase in trap catch as the attractant dispenser dose is increased. Conversely, the (±) disparlure exhibits no clear relationship between stimulus intensity and trap catch (figure 6.4-8). The (−) enantiomer appears to exert little effect on trap catch when added to (+) enantiomer to make a racemic mixture of up to 5×10^{-1} µg of each component. The males that are lured to (±) disparlure deserve special study, for it will be of interest to learn if these males are "phenotypically" different from the
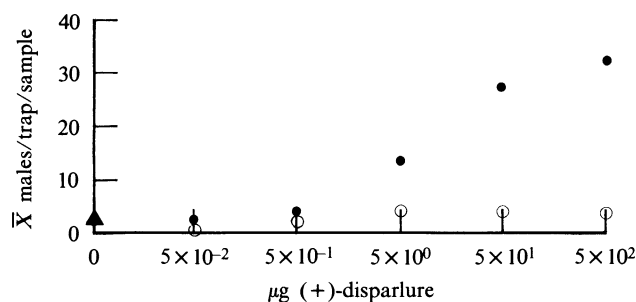
Figure 6.4-8.—*Relationship between trap catch and dispenser dose of* (+) *or* (±) *disparlure. Solid circles denote* (+) *disparlure* (*Iwaki et al. 1974*) *and open circles denote* (±) *disparlure* (*Farchan Chemicals, 9 percent* (±)*-trans-disparlure*) *at twice the dispenser dose of the* (+) *disparlure treatments. An unbaited trap catch is represented by the solid triangle. Experimental methods in Cardé et al.* (*1977c*).

male population more readily lured to (+) disparlure. It has been pointed out for another moth species that attraction to different component blends alone cannot be used as evidence for the existence of different male phenotypes (Cardé et al. 1976).

Lymantria monacha coexists widely with the gypsy moth in Eurasia, and *L. monacha* has been reported lured about equally as well to (+) disparlure and its racemate (Klimetzek et al. 1976, Vité et al. 1976). The emission of (±) disparlure by *L. monacha* females could account for part of the male gypsy moth's lessened response to female *L. monacha* (Schwinck 1955) and reduce chemical communication interference between these sympatric species.

Another antagonist of attraction is the olefin, 2-methyl-*cis*-7-octadecene, which is present in the female gypsy moth (Bierl et al. 1970) and which is evidently biosynthesized to disparlure (Kasang et al. 1974). This compound diminishes greatly the trap catch elicited by (±) disparlure or a virgin female (Cardé et al. 1973) when emitted from the same locus. The olefin also diminishes the trap catch elicited by (+) disparlure, but to a lesser degree than simultaneous emission of the (−) enantiomer (Cardé et al. 1977*b*, Miller et al. 1977). The olefin has been reported to elevate the number of males in a 0.01-ha forest plot

when it was emitted from point sources (Cardé et al. 1975*b*), so that its behavioral effect seems partially dependent upon the context of presentation.

Neurophysiological Perception of Odors by Males

Because (+) disparlure elicits a positive trap catch, and both (−) disparlure and olefin act as antagonists, the peripheral processing of chemical specificity has been the subject of electrophysiological investigations at the whole antennal (Yamada et al. 1976, Miller et al. 1977, Schneider et al. 1977) and single cell (Schneider et al. 1977) levels. Cardé et al. (1973, 1975*b*) suggested that the structural similarity between disparlure and olefin indicated that these two compounds could affect the same receptor, with sensory discrimination dependent upon each compound evoking a unique spike pattern. Schneider et al. (1974) interpreted this hypothesis as a "blocking" of the receptor, consistent with a labeled-line interpretation of sensory processing. (In a labeled-line system, the firing of disparlure receptors over the basal firing rate would be processed centrally as disparlure stimulation regardless of the chemical stimulus.) Indeed Schneider et al. (1977) had "hoped to find a low number of very defined types of receptor cells either responding only to the olefin (and not also to disparlure as do the majority of cells) or at least responding better to the olefin than disparlure." However, they found that, of 100 receptor cells studied, none fitted either of those criteria and neither stimulus elicited a unique spike pattern.

Miller et al. (1977) studied the electroantennogram (EAG) responses of gypsy moths to disparlure enantiomers and the olefin and employed a differential receptor "saturation" technique to gain insight into the number of receptor site types. They suggested that input from (+) disparlure originates largely from one receptor site type, whereas input from the (−) enantiomer and olefin comes largely through another cell type. These findings seem consistent with the similarities in behavior evoked by the two antagonists.

Mechanisms of Communication Disruption

The evidence and methods for disruption of attraction and mating in the gypsy moth by atmospheric permeation with (±) disparlure are reviewed in The Use of Disparlure to Disrupt Mating (this section). The biological effect of (±) disparlure as a communication disruptant is unquestioned, although the precise degree of reduction in mating of wild females and the subsequent effect on population dynamics remain active areas of research. The actual mechanisms that cause communication disruption remain largely speculative (Cardé 1976, Roelofs and Cardé 1977). Among the possible mechanisms when the synthetic of the natural pheromone is the disruptant are a camouflaging of the boundaries of the natural plume, so that a female's odor trail is no longer discernible; sensory adaptation at the peripheral receptor level (lowered response of the sensory neurons organ as the result of repeated stimulation); central nervous system (CNS) habituation (the relatively persistent decrease in response as the result of repeated stimulation but not due to sensory adaptation or muscular fatigue); antagonistic depression or induction in which one pheromone reaction (such as reversing anemomenotaxis) decreases or increases the likelihood of a subsequent behavioral state (such as vertical flight near a tree) (when one activity ceases, a previously inhibited state may appear with a reduced or elevated intensity (Kennedy 1966)); muscular fatigue; interference with specific junctures in the normal mating sequence (such as landing), perhaps by the presence of behavioral antagonists such as the (–) enantiomer; and antagonistic effects of the (–) enantiomer on the second and third factors listed in this series.

The investigations using (±) disparlure are difficult to interpret in the above framework because a combination of an attractant and an antagonist was employed. The presence of the (–) enantiomer may have disparate effects in different background concentrations of natural pheromone. It is possible that (±) disparlure may be particularly disruptive at specific junctures of the behavioral sequence. For example, the (–) enantiomer, when emitted from the same locus as (+) disparlure, is known to decrease both the time spent in upwind anemotaxis (Cardé and Hagaman 1979, Miller and Roelofs 1979) and the likelihood of landing in the field after upwind anemotaxis (Cardé et al. 1977c); both of these effects could aid in disruption of female-male signaling. Conversely, it could be argued that because (±) disparlure is quantitatively less effective in elicitation of some behaviors, it would be less apt to cause CNS habituation or antagonistic depression. These mechanisms are not mutually exclusive, and it seems likely that several factors contribute to successful communication disruption.

Because new tests for evaluation of various disruptant release matrices are contemplated, it should be emphasized that disruptant formulation screening based on tests in small plots (for example, 0.01 to perhaps 1–10 ha) may be inadequate to demonstrate the degree of disruption that would accrue to a large plot (tens or thousands of hectares). Small plots may be useful for preliminary screening of several formulations prior to comprehensive evaluation, but small plots with a formulation for disruption may also lure many males into the test site (Cardé et al. 1975b), greatly elevating the mating pressure in these plots and allowing comparatively little time for potential disruptive mechanisms, such as habituation or antagonistic induction, to occur.

Richerson (1977) has theorized that (±) disparlure's mode of action as a disruptant may be to "block the initiation of short-range sexual behavior of the males," a concept based on the low proportion of males that was observed to land in (±) disparlure-treated plots. Males held in these plots and later released into (±) disparlure-free plots located and mated with females within short periods, indicating that for this disruptant and atmospheric concentration the male's recovery period to return to apparently normal behavior is short (Richerson 1977).

Conclusion

Shortly after the identification of the gypsy moth's sex pheromone, field tests demonstrated convincingly

that a synthetic attractant could disrupt to a degree this pest's sexual signaling system. Ironically, these early successes relegated basic studies of the chiral nature of the pheromone and the behavioral mechanisms of communication to secondary importance; emphasis was placed upon large-scale demonstrations of disruption efficacy. It was perhaps judged that little needed to be understood of the gypsy moth's communication system, although E. A. Cameron (1973), among others, cautioned against such a parochial approach. However, it is now clear that, unlike conventional pesticides, considerable basic information on mode of action and rate of emission of active ingredient is necessary if pheromones are to be implemented into pest management systems.

Future studies of sexual behavior of the gypsy moth undoubtedly will reveal new mechanisms of communication and certainly these studies will refine or replace the behavioral models proposed herein. It is important to continue definition of the communication system and the precise roles of the olfactory, visual, and tactile cues in modulation of each behavioral state. Further, the interplay between meteorological events and pheromone dispersion is a crucial and largely neglected aspect of pheromone research. Future success in manipulation of the gypsy moth's behavior assuredly rests upon the degree to which the process is understood.

Summary

E. Alan Cameron

Since the publication of *Silent Spring* (Carson 1962), scientists and nonscientists alike have accelerated efforts and pressures to minimize the widespread use of chemical pesticides in the environment. Modification of normal behavior of various species of insects, and particularly of mating behavior, has been one of the alternatives investigated. This interest stems in part, at least, from the rapidly improving technological capabilties that permit isolation, identification, and synthesis of the naturally produced chemicals used by many insects for communication.

In most new fields of endeavor, much of the early work suffers from a lack of adequate basic information. Ultimate goals are often pursued actively but prematurely, before the underlying hypotheses have been tested and verified. Those people demanding solutions to operational problems frequently overwhelm the scientists committed to building a sturdy foundation of knowledge upon which a superstructure of action can then be built. J. W. M Cameron (1973), in assessing the then current situation in insect pathology, had this to say:

Unfortunately there appears to be a developing tendency in the last four or five years to insist on mission-oriented research. While recognizing that unapplied research may appear to be frivolous and of little value, one cannot refrain from concluding this account by referring again to Bassi, to Metchnikov and Krassilstschik, to Forbes and Snow, and to D'Herelle, all of whom tried to apply pathogens without clear understanding of what they were or should be trying to do, and none of whom succeeded in their specific aims. It is to be hoped that the lessons of history will be learned by those who make the management decisions so that they will not sell short the scientists who should and must make the research decisions.

By substituting a few names and replacing "pathogens" with "pheromones," the statement unfortunately is applicable to much of what has transpired in gypsy moth chemical ecology work.

Various roles envisioned for disparlure over the years have been described in the preceding pages, and numerous experiments and field tests that have been undertaken have been discussed. Much has been accomplished, especially by way of better understanding the behavior of the insect. This knowledge of behavior, in turn, guides adjustments in expected use patterns of disparlure. Where a decade and more ago there was naive expectation of rapidly implementing use of broadcast applications of disparlure (or of gyptol or gyplure, previously thought to be the pheromone or its chemical analog) for air permeation and consequent disruption of chemical communication among adults, today certain limitations are recognized. Deficiencies may be in the area of requisite basic knowledge of insect behavior; or in the difficulties inherent in monitoring and evaluating tests in sparse populations; or in the availability of a

formulation that will emit lure at a constant and predetermined rate over a specified period of time; or in our capability to synthesize large quantities of pure optically defined disparlure; or in other critical factors, perhaps not yet recognized, that may work against the successful use of disparlure in field situations. Mating success in the field can be reduced with broadcast applications of disparlure, but it has not yet been demonstrated satisfactorily that this prevention of mating of individual moths is adequate to reduce the population as a whole in the next generation, much less eliminate it entirely.

If disparlure is to be used as part of a program to establish a barrier zone, its use would be restricted to that part of the program directed against the natural spread of the insect; disparlure is unlikely to have a role in a program aimed at prevention of the man-associated spread of the moth. Cameron (1976) raised a number of questions that, he suggested, must be answered before a barrier zone should be implemented, questions such as: What is the maximum adult population density in which disparlure will essentially totally disrupt mating? How far do larvae drift during the wind-borne dispersal phase? Are survey and detection networks extensive and intensive enough to pinpoint the appropriate geographical location of any barrier? Are we prepared to undertake extensive population suppression, probably with chemical pesticides, that would be necessary in areas behind the barrier to relieve population pressures within the barrier zone? Are adequate, persistent controlled-release formulations of the lure available? Are adequate monitoring techniques available for use within the barrier zone to evaluate results? Many of these same questions apply to the concept of retarding the spread of the pest along the "leading edge" of the infestation. While progress has been made in some areas, much remains to be learned before satisfactory answers are provided to all these questions and more. Therefore, any attempts to use disparlure operationally in a barrier zone or in control along the "leading edge" of the general infestation would be premature.

The use of broadcast application of the lure has been envisioned to meet various other program objectives as well (Granett 1976). He suggested that spot infestations outside of the generally infested Northeastern United States could be eliminated, that portions of established infestations could be severely reduced through the use of a pesticide followed by disparlure, that foci of population buildups could be treated early to prevent outbreaks from developing, or that large blocks could be treated to prevent a second year of damage. Because disparlure is safe, specific, and nontoxic, and preliminary studies have demonstrated that it is likely to have little or no effect on the pupal parasite *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) (Cameron and Rhoads 1973) or the egg parasite *Ooencyrtus kuvanae* (Howard) (Hymenoptera: Encyrtidae) (Brown and Cameron 1978), public opposition to large disparlure spray programs should be expected to be minimal. However, we are yet some years from operational use of disparlure in any of these contexts.

The above comments should not be interpreted to mean that no role exists for disparlure in gypsy moth pest management. Indeed, the identified gaps in knowledge of the effect of the lure on insect behavior are all the more reason to continue research and developmental work in carefully designed and executed tests. There is certainly room—and a need— for testing broadcast applications of disparlure within larger strategies of integrated pest management directed at the gypsy moth, especially in situations where populations are sparse. However, very careful planning and the development of clearly defined means of evaluating tests must precede such testing.

One of the persistent problems encountered in attempting to review and assess the current situation with respect to broadcast application of disparlure against the gypsy moth is the diversity of methods that various groups of investigators have used in their tests. Plot size has ranged from fractions to tens of thousands of hectares; traps (of various kinds and capacities) baited with racemic disparlure (with or without a keeper) in a wick (cotton or laminated plastic), or baited with a virgin female moth (from either laboratory-reared or field-developed stock) have been used to estimate "mating disruption"; virgin

female moths (again laboratory-reared or from field-collected pupae) have been tethered or released unfettered to act as monitors; monitor females have been exposed at various ages and for varying periods of time (for example, 2 or 3 days) within the same test; various dispersion patterns of monitor insects have been used; only insects from the naturally occurring infestation have been monitored; insects or traps have been exposed at different heights and on trees of widely differing diameters. The consistent factor has been inconsistency.

A much more serious problem is associated with field tests aimed at disrupting mating. A formulation of the lure that would emit a known and reliably constant rate of disparlure has been lacking. Even under controlled, constant conditions in a laboratory, emission from two microcapsule formulations that were tested varied from day to day, up to a twofold difference (Beroza et al. 1975c). Plimmer et al. (1978) and Caro et al. (1977) have reported great variation in aerial concentration of disparlure emitted from microencapsulated formulations of disparlure applied to a woodland or a grass field. Variation was associated with time of day, days after application, and height above the ground. In particular, a large proportion of the total lure emitted flushed out on the day the material was applied in the field. These results emphasize that, in fact, the various field tests conducted over the years were being run with unknown (and unmeasured) quantities of disparlure in the atmosphere, and cast serious question on the meaning of rates of active ingredient per hectare that are always quoted in reporting tests. In reality, there was probably very little lure in the atmosphere during the great majority of the time during which tests were run. A similar reservation must be expressed relative to amounts of lure in wicks of various types of traps used over the years. Clearly much more effort must be concentrated on formulating disparlure for its several specific potential uses if pheromone studies are to continue effectively.

Webb et al. (Disruption Along the "Leading Edge" of the Infestation, in this chapter) have discussed an unexplained "sterility factor" associated with expo-sure of monitor females to pheromone-laden air in test plots during the years 1975-77. They admit the possibility that this condition may be related to the use of laboratory-reared females as test insects but assume this not to be the case. Data gathered by Cameron (1978) from eggs laid by monitor females obtained from field-collected pupae and used in his 1977 tests (Cameron 1977) do not confirm the existence of a "sterility factor." This important discrepancy must be resolved by carefully designed tests, especially if laboratory-reared insects are to be used in behavioral modification tests.

Not only are clearly defined protocols for evaluation required from a practical research and development standpoint, but they must be established if disparlure is ever to be registered with the Environmental Protection Agency for use in a gypsy moth pest management program. These protocols must also have relevance to natural populations under potential use conditions. Similarly, testing must be carried out initially—or at least validated—in the type of forest situation in which use is envisioned. Scrub oak and pine barrens of coastal New England provide one kind of ecological situation; pure mature white oak stands with canopies more than 30 m high on bottomlands in Pennsylvania and Maryland provide a very different set of circumstances; ridge-top chestnut oaks create yet other conditions. What works in one may or may not work in others. Only adequate replicated tests, evaluated by standardized appropriate methods, will provide needed answers. In view of the established differences between laboratory-reared and wild insects, the latter must be used as the test organism. As much information as possible on the quality and history of the population from which the monitor insects are collected should be obtained and reported. If population quality is demonstrated to be important as a conditioning framework for chemical communication, it is conceivable that appropriate monitor insects would have to come from sparse populations and not the more easily located moderate or heavy populations.

Since individual insects do not occur in nature randomly or uniformly, but rather in clumped

dispersions, exposure of test females should reflect natural situations. And the height above ground at which the monitor females are placed must also encompass most or all of the naturally occurring range. Realistic female population densities must be used, and suitable methods are required to estimate adult male population densities at intervals during the seasonal flight period. Ultimately, tests and results must be associated with the population dynamics of sparse or suppressed populations of the gypsy moth, which itself is a poorly understood component of our knowledge.

Tests in which the object was to disrupt mating through air permeation have been conducted using racemic disparlure. Since (+) disparlure has been shown to be so much more attractive than (±) disparlure, it would seem reasonable to anticipate modified results were (+) disparlure substituted in disruption tests. Data simply are not yet available to indicate the magnitude of change, if any, which could be expected. No one has yet identified additional behavior-modifying chemicals associated with the gypsy moth. Very complex activities take place when the sexes are at close range (about 15–30 cm), and the possibility of additional chemical elements in the communication system cannot yet be dismissed.

Fundamental studies of the physiological mechanism of disruption of chemical communication are needed. Critical atmospheric concentrations of lure to create disruption must be identified, and concomitant progress must be made in developing formulations of the lure which have the requisite emission properties to achieve or exceed these levels. Only as these many facets of the problem begin to be tackled and answers obtained will chances of using disparlure effectively and consistently for air permeation to create mating disruption be enhanced. In the absence of answers to critical questions, only empirical testing can be done, and progress will likely be slow.

Baited traps of one kind or another have long been used in survey and detection programs, and they continue to be the heart of the early warning system employed today to detect general spread of the population or isolated introductions into new areas.

Several attempts have been made to develop methods for employing traps, in grids or otherwise, as a means with which to monitor or predict numbers of insects in a population. Such an ability would have great value in population dynamics studies; it could also aid substantially if it could be incorporated into predictive models which would look ahead to the next year's population. Decisions on treatment could be based on yet another input; regulatory efforts might be redirected to areas of greatest need; and changes in general population levels might be recognized or anticipated earlier than is now possible.

The trap used for survey and detection should be highly efficient: it is important to catch certainly most if not all moths that approach or contact a trap. If traps are ever to be used to eliminate an infestation, they, too, would have to be highly efficient in capturing males. On the other hand, a trap that caught only a fraction—but a relatively constant fraction—of those moths approaching it might find a role in population monitoring. Alternatively, a trap with a very high capacity of moths might serve this latter purpose equally well. Many answers remain to be obtained if the role of traps is to be fully and effectively exploited in the gypsy moth program.

Perhaps the most significant results that have come out of the diverse studies involving disparlure are those associated with behavioral investigations. The concentrated efforts of the 1970's have enhanced understanding manyfold over that of a decade ago. With disparlure attempts have been made to modify behavior. The studies of Doane, Richerson, and Cardé in particular have given a much clearer understanding of just what it is that is being modified. With an increased understanding of behavior, results of empirically designed behavior-modification tests that failed are more easily explained. Yet the surface of the basic knowledge needed has barely been scratched.

The report of the synthesis and activity of optically active disparlure by Iwaki et al. (1974) opened a whole new array of questions on the use of disparlure for all manner of uses in gypsy moth programs. The conclusions drawn from all studies conducted with

(±) disparlure must be viewed in a new light. The attractiveness of (+) disparlure exceeds that of (±) disparlure by an order of magnitude or more, which has obvious implications for survey and detection or for population delimiting, monitoring, or evaluation activities. Behavioral responses of males to (+) disparlure are quite different than to (±) disparlure. Disruption capabilities of broadcast applications of (+) disparlure have not yet been tested. In many ways, late 1974 presented a situation similar to the one existing in 1970: A "new" pheromone had been identified as the pheromone of the gypsy moth, and basic studies needed to be repeated. Only this time it was known that (±) disparlure is a biologically active material; it may well retain a potential role in gypsy moth integrated pest management if only because of its relative ease of synthesis compared to (+) disparlure.

The past is prologue. If we build on the foundation of knowledge developed especially over the last decade, if the necessary basic studies are supported by managers of research dollars, and if scientists are allowed to make the research decisions they must, the statements of J. W. M. Cameron (1973) quoted earlier will no longer seem as appropriate to studies of the chemical ecology of the gypsy moth. If history persists in repeating itself, little useful progress can be expected. The next decade presents many challenges and opportunities; may they be accepted and exploited in the best interests of science.

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6.5 Mass Rearing and Virus Production

Development of Mass-Rearing Technology

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Introduction

The capability for efficient, large-scale production of insects is essential to the development of the currently envisioned integrated pest-management program for the gypsy moth. For, example, large-scale tests to determine the feasibility of using natural enemies such as parasites or pathogens or the sterile-male principle require large numbers of host or test insects.

During the past 2 years, the need for insects for parasite production exceeded 10,000 per day, and nearly 20,000 larvae per day were required for production of the nucleopolyhedrosis virus. Moreover, to carry out laboratory tests involving pheromones, pesticides, and development of rearing technology, an additional 5,000 or more insects per day are often required. Finally, to conduct field tests of the sterile male technique adequately, it may be necessary to produce 100,000 or more insects daily.

However, the high cost of rearing ($90 per thousand, Secrest and Collier 1967) and problems with insect quality have been major factors limiting the development of desired biological and autocidal control measures.

Although techniques for rearing the gypsy moth have already been developed (Leonard and Doane 1966, Magnoler 1970a, Odell and Rollinson 1966), these are feasible only for small-scale laboratory rearing. Information on advances from small-scale to larger scale rearing techniques is lacking, although several improvements were made at the Animal and Plant Health Inspection Service (APHIS) Gypsy Moth Methods Development Laboratory, Otis Air Force Base (Secrest and Collier 1967, Tardif 1975). Undoubtedly, other unpublished improvements in production efficiency may have been made at various other locations where gypsy moths are frequently reared.

In spite of improvements in rearing up to 1975, production costs were still in the range of $60 to $80 per 1,000 insects. Obviously, this cost was rather high when compared to that of other lepidopterous insects currently being mass reared. For example, the tobacco budworm had been reared for less than $10 per 1,000 (Raulston and Lingren 1972). In view of the low-cost techniques available for mass rearing various agricultural crop pests, it seemed likely that similar significant improvements could also be made in rearing the gypsy moth.

Consequently, in 1975, a program was initiated at Otis AFB to improve the technology for mass rearing the gypsy moth. A team of two research entomologists with previous experience in insect rearing and an agricultural engineer joined the Expanded Gypsy Moth Program at Otis to implement the needed research and development in cooperation with other U.S. Department of Agriculture personnel.

The ultimate goal of this research was the development of an optimal system for mass rearing the gypsy moth. The more specific objectives included the development of low-cost diets and containerization, an optimal rearing environment, methods for shortening or preventing diapause, methods for handling of insect stages, procedures for control of disease and incidental contaminants, quality-control procedures, and pilot-scale facilities for insect and virus production. Another major objective in the overall program—the development of a more efficient system of production of virus—is discussed in the second part of this chapter. The status of progress on accomplishment of many of these objectives is the subject matter for this section.

Test Insect Populations

For conducting laboratory and field experiments it is important that the insects used as test populations are healthy, vigorous, and capable of performing in a reasonably uniform manner. In the past, variations in the quality of wild and/or colonized gypsy moths over periods of time and from one location to another have

been particularly troublesome to many investigators. Such variations may be due to disease, genetic changes, or various unknown physiological disorders. Undoubtedly, differences in the development of gypsy moths reared on artificial diets at various laboratories can be partly explained by possible differences in the quality of the test insects involved. Although all the factors that cause variation in population quality may not be understood, the use of methods described here can insure that test populations are reasonably healthy and vigorous.

Quality Variation in Wild Populations

Several problems were encountered in obtaining good wild stock for test populations. As mentioned previously, the state of health or vigor of such populations varies with location and time. Also, wild stock can be used for only a portion of the year, from January through July, because of the relationship between proper chilling of the eggs to terminate diapause and promote the desired synchrony of hatch.

Moreover, in 1976, wild stock collected from a moderate infestation near Dighton, Mass., turned out to be loaded with virus and lacking in vigor. In 1977, wild stock collected from Stroudsburg, Pa., was found to be relatively free of virus and performed reasonably well in the laboratory. Contrary to expectations, the Stroudsburg field population was virtually eliminated by virus later in 1977 and could not be used as the source of wild stock for 1978. In 1978, it was decided to take a different approach in obtaining wild stock for laboratory studies—that is, egg masses would be collected from several geographical areas and evaluated in the laboratory for relative performance. This action was prompted because of the need for vigorous wild test stock for use in laboratory experiments and for use in field tests to determine the feasibility of using the sterile-male technique for suppression of the gypsy moth.

Evaluation of Performance of Wild Strains

Methods

In January and February 1978, egg masses were collected from low to moderate infestations in New

Jersey, Pennsylvania, and Massachusetts and refrigerated in the laboratory at 5°C. Various measurements were made, including incidence of egg parasitism, percent fertile eggs, egg hatch, weight of eggs and egg masses, levels of virus associated with egg masses, and rate of larval growth on artificial diet in the laboratory.

To determine the natural incidence of virus, each of 10 egg masses from each strain was divided into two parts: One was surface disinfected in 10 percent formalin as described by Shapiro (1977), and the other was left untreated. Formalin was used as a disinfectant because Shapiro found that it was not only a more effective viricide than the previously used sodium hypochlorite but was also highly effective when applied to intact egg masses. Larvae from the untreated portions of egg masses were reared both aggregately and individually on artificial diet and observed for virus-induced mortality, a method similar to that reported by Doane (1969, 1975). To determine growth rates, 480 larvae per strain were removed from the disinfected portions of egg masses, reared on modified tobacco hornworm diet (table 6.5-1), and weighed at 7, 14, and 21 days of age. After 21 days, a judgment could be made regarding the relative vigor of the strains (table 6.5-2).

Table 6.5-1.—*Modified tobacco hornworm diet used for rearing the gypsy moth*[1]

Ingredients	Amount per liter
Wheat germ, raw	80.0 g
Casein, industrial grade, 80% protein	36.0 g
Sucrose	32.0 g
Torula yeast	16.0 g
Salt mixture	8.0 g
Vitamin mix[2]	10.0 g
Cholesterol[3]	0.2 g
Methyl paraben	1.0 g
Sorbic acid	2.0 g
Agar	20.0 g
Linseed oil, raw[3]	1.0 ml
Water	800.0 ml

[1]Modified from the diet developed by Yamamoto 1969; cost per liter=$0.50–$0.60.
[2]Vitamin mix #26862 (Roche Chemical Co.) includes B-vitamins, ascorbic acid, choline chloride, and inositol.
[3]For virus production, cholesterol and linseed oil may be omitted.

Table 6.5-2.—*Assessment of suitability of candidate wild populations of the gypsy moth for laboratory colonization*

Population origin	Percent parasitized eggs	Percent hatch	Percent larval mortality from NPV[1]		Larval weight at day 21[2]	
			Untreated	Treated	Males	Females
Yards Creek, N.J.	24	68	2	0	0.57 g	1.2 g
Bridgewater, Mass.	15	67	8	0	.43	.90
Bald Eagle, Pa.	33	67	13	0.3	.51	.92
Ferrandsville, Pa.	21	71	21	0	.47	.97
N. Ferrandsville, Pa.	19	67	28	0.7	.40	.85

[1]Virus-induced mortality after 21 days in larvae reared from untreated egg masses; mortality in larvae from formalin-treated egg masses was recorded during the entire larval developmental period; insects were aggregately reared at eight larvae per ME-6R container.
[2]Individually reared in 45-ml polystyrene cups and transferred to fresh diet after 14 days.

Results

No differences were found among the various wild populations in percentages of embryonated eggs, egg hatch, or in weight of the eggs. Incidence of egg parasitism showed no relationship with population vigor. However, larvae from egg masses with the least incidence of virus were generally the most vigorous, as indicated, for example, by the greater weight of the 21-day-old larvae of the Yards Creek, N.J., population. On the basis of this data, the Yards Creek population was selected for laboratory propagation and as the parental (P) and F_1 stock for laboratory and field tests for the sterile-male project.

Rate of Increase as an Index of Population Perfomance

Although the wild stock for colonization had been selected on the basis of incidence of virus in egg masses and growth rates of test populations, it was decided that a better index of overall performance could be obtained by measuring the innate capacity for increase of the various wild populations.

Methods

A total of 160 insects from each wild stock was removed at random from formalin-disinfected egg masses, placed on modified hornworm diet at 8 larvae per ME-6R cup (fig. 6.5-1, table 6.5-3), and reared at 25° C, 60 percent relative humidity. All insects were provided with fresh diet at 14-day intervals (at 14 and 28 days after initial placement on diet). All of the adult females that emerged from each population were individually mated to determine the percentage capable of reproduction.

Calculation of Ri

The calculation of the rate of population increase (*Ri*) per generation as used here was modified from that developed by Birch (1948). The mean generation time (*T*) used by Birch was not used in the calculations here since the gypsy moth has only one generation per year in the field and no appreciable differences in the development rate of the various wild populations were observed in the laboratory. Also, the final data are expressed as the potential number of F_1 offspring (males and females) produced per female instead of the number of females only as used by Birch. It is believed that meaningful comparisons of population performance can be made with the simplified method used here.

The potential rate of population increase (*Ri*) per female per generation in laboratory-reared gypsy moths was calculated after having first determined the percentage of initial female larvae that survived to the adult stage and were capable of reproduction. For example, if 200 newly hatched larvae were placed on diet, then the initial number of females was 100 (a

50:50 sex ratio is assumed, although this is not always the case). If 90 percent survive to maturity and subsequent individual pair matings show that 90 percent of the mated females lay viable egg masses, then the percent of reproductive adults is 0.90×0.90 or 81 percent.

The potential Ri is then obtained by multiplying the $\bar{x}$ eggs per female by the percentage of females that was reproductive. Thus, if the $\bar{x}$ eggs per female is 700, the potential Ri is $700 \times .81$ or 567-fold increase. An easy, fairly accurate way to determine the number of eggs per mass is to obtain the weight of the egg mass in

Figure 6.5–1.—*Containers used for individual and aggregate rearing of the gypsy moth*: A, *45-ml styrene cups (Thunderbird Plastics)*; B, *180-ml m-trene® ME-6R*; C, *fluted XE-6 cups (Sweetheart Plastics)*; D, *500-ml Dixie® cups.*

Table 6.5–3.—*Containers and closures used in rearing the gypsy moth*

Stock no.	Description	Source
2186SE	Dixie®, heavy-duty paper, 500 ml	American Can[1]
3086G	Plastic closures for 2186SE	American Can[1]
2168SE	Dixie®, heavy duty squat, 250 ml	American Can[1]
3068G	Plastic closures for 2168SE	American Can[1]
23ST	Paper cups, condiment,52.5 ml	American Can[1]
ME–6R	M-trene® plastic cups, 180 ml	Sweetheart Plastics[2]
XE–6	Polyethylene food cups, 180 ml	Sweetheart Plastics[2]
AS306	Paper lids for ME–6R and XE–6	Sweetheart Plastics[2]
250	Styrene cups, 45 ml	Thunderbird Plastics[3]
	Paper lids, 1.476 in. diameter	Standard Cap & Seal[4]

[1]American Can Company, Dixie® Marathon® Products, Greenwich, Conn.
[2]Sweetheart Plastics, Inc., Wilmington, Mass.
[3]Thunderbird Container Corp., Box 12033, El Paso, Tex.
[4]Standard Cap & Seal, Box 80336, Chamblee, Ga.

milligrams and then multiply by a factor of 1.33. The actual Ri is subsequently obtained by multiplying the potential Ri by the percentage egg hatch.

Results and Discussion

According to the Ri values, the Yards Creek population showed the highest potential for increase, although the Bridgewater stock was probably not significantly different (table 6.5-4).

Except for a slightly longer developmental time (3-4 days), overall performance of the Yards Creek and Bridgewater stock was similar to the control (NJF$_{16}$) colonized strain.

Perhaps the most significant factor determining the performance of the populations derived from wild egg masses was the associated incidence of virus. The Yards Creek, N.J., and Bridgewater, Mass., populations both had a low incidence of virus and showed a high capacity for increase, while those collected from Pennsylvania, particularly the Ferrandsville North population, had a higher incidence of virus and a much lower potential for increase.

Although all egg masses from which the test populations originated were rigorously disinfected with formalin, virus-induced mortality was observed in a few nearly mature female larvae in the Bald Eagle and Ferrandsville North populations. Although the percentage of known virus-induced mortality was low (3 of 320 insects), it appeared that, at least in these populations, some virus had survived the disinfection procedure. Because virus-induced mortality occurred in some larvae, it was suspected that the lower rate of increase of the Pennsylvania populations may have been due to sublethal virus infections—that is, sublethal in the larval stage. Also, the higher incidence of pupal mortality observed in the Bald Eagle and Ferrandsville North populations may have been caused

Table 6.5-4.— *Performance of various wild populations of the gypsy moth for laboratory propagation*

Performance criteria	Population origin[1]					
	NJF$_{16}$	YC	BW	F	BE	FN
Pupal weight (grams)						
Males	0.70	0.54	0.53	0.47	0.44	0.43
Females	2.2	2.3	2.1	2.0	2.0	1.8
Days to adult						
Males	41	45	45	45	45	46
Females	43	46	47	48	47	48
Survival (percent of N infested)						
Male pupae	39	43	43	44	45	46
Female pupae	60	48	45	48	45	38
Total	99	91	88	92	90	84
Male adults	39	43	41	43	44	46
Female adults	56	45	43	44	38	29
Total	95	88	84	87	82	75
Reproduction						
Reproductive						
Females (percent)	93	88	82	76	71	48
$\bar{x}$ eggs per female	867	870	867	687	801	644
Ri per female[2]	806×	766×	711×	591×	569×	309×

[1]NJF$_{16}$ = New Jersey (colonized in the laboratory for 16 generations); wild strains derived from egg masses collected from YC = Yards Creek, N.J.; BW = Bridgewater, Mass; F, BE, and FN = Ferrandsville, Bald Eagle, and North Ferrandsville, Pa., respectively.

[2]Ri = potential rate of population increase from the parental (P) to the F_1 generation. Actual rate of increase determined by multiplying Ri by percent hatch.

by virus but the cause of death was not determined.

It appears that the virus, although present in levels that are sublethal to larvae, may nevertheless have a considerable impact on the population by reducing the reproductive potential. For example, the Yards Creek population that had the least incidence of virus, according to bioassay, showed a potential rate of increase that was 2.5 times that of the Ferrandsville North Stock, which had the highest level of virus. The possibility that sublethal virus levels may result in population reduction by decreasing the reproductive potential is being given further study.

Lewis (1961) emphasized the importance of collecting egg masses for laboratory colonization from areas free of obvious virus disease. However, as shown in this study, there is a high degree of variation in incidence of virus and consequently in relative vigor among populations before the disease becomes really obvious in the field. Thus to insure that laboratory test stock or colonies are disease free and vigorous, populations should be collected from several prospective areas and evaluated for overall performance.

It is also conceivable that such population performance evaluations may be carried out for the purpose of selecting suitable test-plot areas for conducting field trials. With a reliable quality index of the field populations, results of field tests may be more conclusive than in the past.

It is hoped that by the end of this study a quality index based on the capacity for population increase will have been developed, to be used as a reliable measure of the overall performance of wild and laboratory colonized populations.

Colonized Strains

It is not yet known to what extent gypsy moths that have undergone prolonged colonization in the laboratory differ from wild stock. Richardson and Cameron (1974) reported that rates of pheromone emission in laboratory-reared females were considerably less than that of wild stock. However, the question still lingers as to whether the laboratory-reared stock used in the tests was reasonably healthy and vigorous.

It is expected that adaptive changes arising within populations subjected to laboratory colonization over prolonged periods could lead to significant genetic and therefore physiological and behavioral modifications. A large effort is being made at this facility to determine specific differences between colonized and wild insects that may have considerable impact on their use in a sterile-male release program. In the meantime, both colonized and wild stock are being used in experiments involving the development of rearing technology.

Several populations of gypsy moths collected from diverse geographical areas have been colonized over the past several years at the APHIS rearing facility at Otis AFB for as many as 17 generations. Although data are lacking on the comparative behavioral performance of the various colonized strains, there appears to be little difference in developmental performance in the laboratory. However, since a New Jersey (NJF_{15-17}) strain was available in large numbers throughout the year, it was adopted as the colonized strain for subsequently described experiments.

Sex Ratio vs. Hatch Time

Evidently wild populations may differ not only in incidence of disease and overall vigor but also in sex ratio and sex differences that are related to time of larval hatch (Leonard 1968). Such differences may show up in colonized populations as well. A study of this behavior was initiated to determine if predominantly male larvae could be obtained to provide production stock for the sterile-male program. The potential advantages in terms of reduced stock for the sterile-male technique are considerable. Females, in contrast with males, consume three to four times as much diet and require proportionally more container and shelf space for rearing, not to mention the additional labor cost of rearing the unneeded females.

To date, studies have concentrated in three areas: Determining the extent of sexual differentiation in time of hatch in colonized vs. wild strains; determining the degree of natural variation among individual egg masses; and exploiting the sexual differentiation phenomenon for mass production of the

desired sex. It is worth mentioning here that development of techniques for production of predominantly female larvae is also a desired goal because it has been determined that females produce two to three times more virus than males (Shapiro 1977).

Methods

To determine sex differences in time of hatch, egg masses were selected at random, individually dehaired, and surface disinfected with 10 percent formalin. Generally, 50 or 100 eggs were randomly removed from each mass and placed in individual 30-ml plastic cups. Those larvae that hatched each day were removed and placed on diet in XE–6 containers at 10 per cup. Larvae were reared to the final larval instars (usually 26 to 28 days after initial placement on diet) and the number of males and females was tallied.

Results and Discussion

The hatching profiles in terms of sex ratio vs. time of hatch for a colonized and two wild populations are shown in figure 6.5–2. The colonized (NJF$_{15}$) and the Stroudsburg wild population showed a predominance of females hatching during the first 2 days of the hatch period; on the third day and beyond, most of the larvae were males. Although the overall sex ratio approached 50:50, during the latter half of the hatching period (after the second day), the sex ratio was about 60:40 in favor of males. These results were similar to these reported by Leonard (1968). Although the data are not presented here, considerable variation was noticed between egg masses; in some the ratio exceeded 80:20 during the latter half of the hatching period. However, in other cases, the sexual pattern is reversed and males predominate during the early hatch period. This matter should be pursued further to determine the potential for selection of strains with the desired hatching patterns.

Considerable differences in sex vs. time of hatch occurred in wild populations, which may be useful as an indication of population quality. For example, egg masses collected from Dighton, Mass., in 1976 yielded a preponderance of males throughout the hatching period (the overall sex ratio was 60:40).
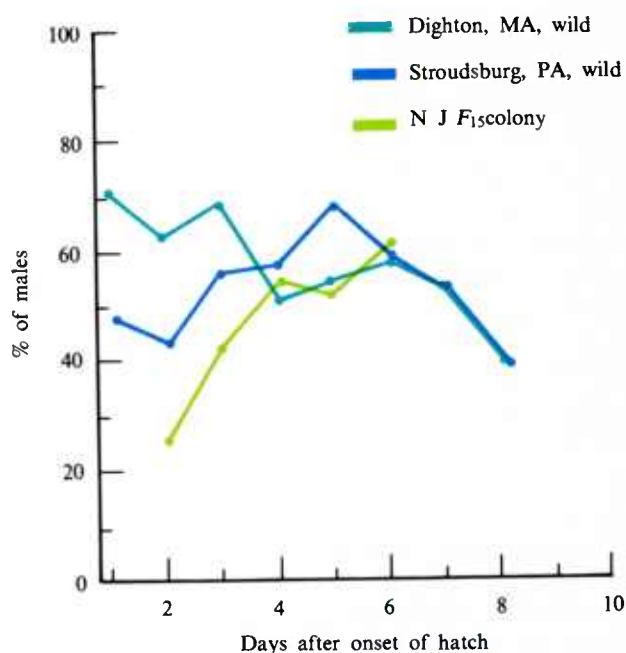
Figure 6.5–2.—*Relative percentage of males in relation to time of hatch in wild and colonized gypsy moths.*

The Dighton area was heavily infested with gypsy moths that had suffered high mortality from virus and parasites. By contrast, egg masses collected near Stroudsburg, Pa., yielded sex ratios and a sex vs. time of hatch profile similar to that of the New Jersey colonized strain (fig. 6.5–2). The Pennsylvania strain was taken from an area with low virus infection and little parasitism. The Dighton strain showed poor growth on artificial diets, whereas the Pennsylvania strain showed excellent growth response.

Sex Ratio Distortions

According to earlier investigators, sex ratio distortions are not uncommon in natural gypsy moth populations. Campbell (1963a,b) found that disease and desiccation caused a greater mortality in females because of the longer larval development period. Usually the males, by pupating earlier, were able to escape because they were not exposed to these elements long enough to cause mortality.

Further evidence that sex ratio distortions are caused by differential mortality of the sexes rather than sex distorting genetic or physiological mechanisms was provided by subsequent investigations. Thus, Campbell (1967) examined eggs and first-instar larvae from several wild populations and found no significant deviation from the expected 50:50 sex ratio. Similar observations were reported by Leonard (1968) on two different populations over a 2-year period. The observed preponderance of males in the 1976 Dighton population may have resulted from differential mortality of the two sexes. Thus, more females died during larval development than males. This phenomenon also occurred in the Ferrandsville North population (table 6.5-2), in which there was a relatively higher incidence of virus. However, even when differential mortality is taken into account, the sex vs. hatch time pattern in the Dighton population still differs from that of the Stroudsburg wild and New Jersey colonized populations. Because of the obvious implications of these findings in the development of a system for rearing predominantly males or females, this area should be given further study.

From the studies conducted to date, the prospects of rearing a predominantly male or female population by infesting larvae that hatch either earlier or later in the hatch period appear encouraging. It is conceivable that techniques may be developed wherein two-thirds or more of the insects would consist of either sex.

Development of Diets and Containerization

Several artificial diets have been developed for laboratory rearing of the gypsy moth (Leonard and Doane 1966, Magnoler 1970a, ODell and Rollinson 1966, Ridet 1972). Essentially, the diets were modifications of the wheat germ-casein formulations developed by Vanderzant et al. (1962) for rearing the cotton bollworm, *Heliothis zea* Boddie.

Up to now the primary diets used for larger scale rearing of the gypsy moth were the Bioserve and APHIS modifications of the ODell and Rollinson formulation (Bioserve, Inc., Frenchtown, N.J., and APHIS, Otis AFB, Mass.). However, both diets not only contain numerous ingredients but are rather expensive and laborious to prepare.

For these reasons research was initiated to find or develop a diet that is less expensive and provides optimal nutrition. During the course of these investigations, several diets previously developed for rearing the gypsy moth and other lepidopterous insects were evaluated. Diets that showed the most promise were further modified and evaluated. Several alternative, low-cost, nutrient sources, gelling agents, and microbial inhibitors were tested. The effects of pH on the diet and temperature during diet preparation were also investigated. Finally, some attempts were made to develop a chemically defined diet. Because the methods used for these studies differ from those reported by other workers, a brief description is given here.

Methods and Materials

Test Insects and Disinfection

Insects used in these studies were derived either from field-collected egg masses or from laboratory colonized stock (New Jersey F_{15-17}). Intact (nondehaired) egg masses were surface disinfected in 10 percent formalin for 1 hour followed by a similar period of rinsing in cold tap water (Magnoler 1970a, Shapiro 1977).

Diet Preparation

Several methods for preparing diet have been used with variable success. Research is in progress to determine the optimal methods of diet preparation, but the methods described here have been tentatively adopted as the standard procedure.

Dry ingredients, except agar, are weighed out, combined in plastic bags, sealed, and stored in a freezer (-15° C) until needed. Liquid ingredients (oils, vitamin solutions) are stored in the refrigerator and measured out during diet preparation.

Tap water is heated to boiling and poured into a prewarmed 3.8-l blender. The blender is equipped with a variable speed transformer to regulate mixing speed. With the blender operating at low speed, agar is

blended in, followed by the remaining dry and liquid ingredients. After blending for 60 seconds at low speed, diet is dispensed immediately into containers. Care is taken to avoid vortexing during the mixing process to minimize entry of air into the diet. Certain exceptions to this procedure will be noted later.

Handling of Stages

Newly hatched larvae were placed in groups of eight per ME–6R or XE–6 cup (fig. 6.5–1, table 6.5–3), containing 75 to 90 ml of diet and reared at 25° C, 60 percent relative humidity. Insects were usually transferred to fresh diet after 21 days and then reared to pupation either in ME–6R or XE–6 cups or in Dixie® cups with clear plastic lids (fig 6.5–1).

After pupation was completed, pupae were sexed and transferred to 500–ml cups for adult emergence. The first 10 females to emerge in a given treatment were individually mated in the cups. Egg masses were either scraped or left attached to a cutout section of the container and incubated at 25° C for 28 days. Thereafter the egg masses were chilled for a minimum of 3 and a maximum of 6 months at 5° C before incubation at 25° C to determine hatch. Samples of 25 eggs per mass ($N = 10$ egg masses per treatment) were used to determine the incidence and duration of hatch.

Results and Discussion

Evaluation of Various Diets

Several low-cost diets used for rearing other lepidopterous insects were compared with formulations specifically developed for rearing the gypsy moth (table 6.5–5). The diets evaluated contained wheat germ and casein, wheat and soybean flour or dried beans, and torula yeast as the principal sources of nutrients.

Of the various diets tested, overall survival and performance were better on the tobacco hornworm formulation (table 6.5–1), which was modified somewhat from the Yamamoto (1969) formulation. Although the gypsy moth could be reared on the bean-based diets, the overall development and

Table 6.5–5.— Various artificial diets evaluated for mass rearing the gypsy moth

Wheat germ/casein formulations	Cost per liter[1]
ODell and Rollinson (1966)	—
ODell and Rollinson APHIS modified	1.02
ODell and Rollinson Bioserve modified	—
Leonard and Doane (1966)	—
Magnoler (1970)	.62
Yamamoto (1969) modified	.52
Wheat/soybean and/or bean-based diets	
Burton and Perkins (1972) wheat/soy blend	.34
Shorey and Hale (1956) lima bean	.40
Patana (1969) pinto beans	.33

[1]Although the data are not given here, the cost of the original ODell and Rollinson diet and the Bioserve formulation would probably fall between that of the APHIS and Magnoler diet. The Leonard and Doane formulation would cost approximately the same as the Magnoler diet.

survival were poor in comparison with the wheat germ/casein diets. Several modifications of the lima bean diet and wheat/soy blend diet were tested but none proved to be as satisfactory as the hornworm diet; thus, it was adopted as the standard with which other subsequently tested formulations were compared.

All of the wheat germ/casein formulations previously developed for rearing the gypsy moth supported reasonably good growth and development but had to be ruled out as potential diets for mass rearing because of their overall greater cost. However, because the ODell and Rollinson (Bioserve modification) and the Magnoler diets gave somewhat better results in these tests, they were evaluated in several subsequent experiments.

Development of More Simplified Diets

Results of preliminary experiments suggested that certain ingredients in the modified hornworm diet could be deleted without adversely affecting development of the gypsy moth. Such nonessential ingredients included cholesterol and linseed oil (source of linoleic acid), raw sugars (carbohydrate source), and torula yeast. Sufficient sterols and fatty acids as well as carbohydrates can be supplied by wheat germ if present in the diet in sufficient quantity. Although torula yeast is an inexpensive source of B-

vitamins and a protein supplement, sufficient vitamins were already supplied by the vitamin mix. Also, adequate protein was available in the wheat germ and casein.

Thus a simplified wheat germ/casein diet (table 6.5-6) was made by removing the cholesterol, linseed oil, yeast, and sucrose provided as supplements in the hornworm diet. Increasing the level of wheat germ from 8 percent (as in the hornworm diet) to 12 percent compensated for the loss in bulk caused by deletion of the other ingredients. Moreover, the increase in raw wheat germ content provided additional sterols and essential fatty acids. The most significant benefit from increase in wheat germ content was the change in the gelling properties of the diet—the agar content of the diet was reduced by 25 percent and still produced a satisfactory gel. These changes along with a 30-percent reduction in the casein level resulted in the development of a greatly simplified larval diet that could be formulated for nearly half the cost of the modified hornworm diet.

Developmental and reproductive performance of a wild population of gypsy moths reared on the simplified high wheat germ diet compared favorably with other formulations tested, including the standard modified hornworm diet (table 6.5-7). However, the best overall performance as judged by survival and reproductive potential was on the modified horn-

worm diet without cholesterol and linseed oil. The unusual mortality of larvae grown on the Magnoler and modified hornworm diet (C and E, table 6.5-7) was probably due to poor quality linseed oil. Unless it is kept tightly sealed and refrigerated, raw linseed oil undergoes rapid oxidation and becomes rancid. As indicated in this experiment, the addition of supplemental fatty acids to diets containing 8 percent or more of raw wheat germ seems unnecessary and may even do more harm than good.

It is concluded from this and similar experiments that the high wheat germ formulation is an economical and nutritionally adequate diet for mass rearing the gypsy moth, particularly for virus or sterile-male production. For colony maintenance, the modified hornworm without linseed oil and cholesterol may be preferred. Both the modified hornworm and the high wheat germ diet have been used as colony maintenance and production diets for nearly 2 years now with good results.

The development and reproductive performance of wild populations of gypsy moths reared on the modified hornworm and high wheat germ diets are compared with results obtained by other workers (table 6.5-8). The comparisons show that the performance of the gypsy moth reared on the inexpensive high wheat germ diet compares quite favorably with the best results reported by other workers. An excellent study by Hough and Pimentel (1978) in which gypsy moths were reared on various kinds of foliage at 24° C permitted a meaningful comparison of development on artificial diet with development on the natural host plant. The data show that development and reproduction of gypsy moths reared on the hornworm and high wheat germ diets are very similar to that of wild stock reared on white-oak foliage.

Whereas these comparisons seem to imply that the artificial diets are nutritionally equivalent to oak foliage, it would be necessary to rear gypsy moths on both artificial and natural diets for two or three generations before the question of nutritional equivalence could be answered. Also, it is not altogether meaningful to compare data obtained on

Table 6.5-6.—*Composition of high wheat germ diet used for rearing gypsy moths[1]*

Ingredients	Sources	Grams per liter
Wheat germ, raw	Niblack Foods; Mennel	120
Casein	Milk Specialties	25
Salt mix, Wessons	ICN Pharmaceuticals, Inc.	8
Sorbic acid	ICN Pharmaceuticals, Inc.	2
Methyl paraben	Tenneco Chemicals	1
Vitamin premix #26862	Roche Chemicals	10
Agar[2]	Moorhead and Co.	15
Water		800 ml

[1]Cost per liter=$0.33; with HWG=$0.24.
[2]Gelcarin HWG (Marine Colloids) at 1 percent (10 grams per liter) may be used as an agar substitute.

Table 6.5-7.—*Development of the gypsy moth on various artificial diets (Pennsylvania wild strain)*[1]

	Diets[2]				
	A	B	C	D	E
Weight (grams)					
Pupal weight of males	0.52	0.39	0.47	0.59	0.52
Pupal weight of females	2.14	1.50	1.77	2.71	2.35
Developmental time					
Days to adult,male	47	46	52	47	48
Days to adult, female	49	50	54	50	50
Survival (percent)					
Pupal yield	95	85	76	96	83
Adult yield	91	84	64	91	77
Normal males	95	91	100	93	100
Normal females	91	100	90	100	97
Reproduction					
Reproductive females (percent)	82	84	58	91	69
Eggs laid per female	840	643	732	1084	925
Ri (potential)	689×	540×	425×	986×	638×

[1]Reared at eight larvae per ME-6R container; diet changed when larvae were 21 days old.
[2]A = High wheat germ diet (see table 6.5-6.)
B = Bioserve modification of ODell and Rollinson (1966) diet.
C = Magnoler (1970) diet.
D= Modified hornworm without cholesterol or linseed oil.
E = Modified hornworm (see table 6.5-1).

Table 6.5-8.—*A comparison of the performance of wild strains of the gypsy moth reared on various diets in the laboratory*

Food source	Average pupal weight (g)		$\bar{x}$ Developmental time[1] (days)		Percent adult yield[2]	Average number of eggs per female
	Males	Females	Males	Females		
Oak foliage[3]	0.64	2.0	47	48	71.0	919
Magnoler diet[4]	.57	2.3	51.4[5]		96.8	865
Hornworm, modified[6]	.59	2.7	47	50	91.2	1084
High wheat germ[6]	.52	2.1	47	49	91.2	838
ODell and Rollinson[4]	.46	1.3	48.9[5]		77.8	442
ODell and Rollinson (1966)	.62	2.0	—	—	—	—
Leonard and Doane (1966)	.43	1.6	—	—	50.8	750
Field-collected pupae[4]	.49	1.4	—	—	41.3	—
Field-collected pupae[7]	.50	1.4	—	—	—	—

[1]From hatch to adult eclosion.
[2]Adults recovered from initial larvae infested.
[3]Hough and Pimentel (1978); reared on white-oak foliage.
[4]Reported by Magnoler (1970).
[5]Data are for both sexes combined.
[6]See formulations, tables 6.5-1 and 6.5-6.

insects reared in the laboratory with that of insects grown on natural host plants in the field unless it can be assured that the latter are relatively free of disease and natural enemies and not limited by quantity of food. It is reasonable to expect to be able to rear larger, faster developing, and more fecund insects in the laboratory than are generally found in the forest, where development is restrained by disease, parasitism, unfavorable weather, and a host of other natural calamities.

Effect of Diet Change

Previous investigators, whether involved in small- or large-scale rearing, have found it necessary to provide fresh diet one or more times during the course of larval feeding. Because of the rather prolonged feeding period of gypsy moth larvae, particularly the females (nearly 5 weeks at 25° C), the diet must either be exceptionally stable with regard to its nutritional and physical properties or replaced with fresh diet as necessary.

The necessity for replacing the diet not only increases labor costs but makes it difficult to apply conventional automated rearing technology. For example, technology is available for automated implanting of eggs and even for harvesting pupae, but a system for providing fresh diet to larvae adds a new and more costly element to insect mass rearing.

The problems are illustrated by data presented in table 6.5-9. The Ferrandsville, Pa., wild stock was aggregately reared on the high wheat germ diet at eight larvae per cup. One group (80 larvae) was permitted to develop to pupation without a change in diet; a similar group was transferred to fresh diet after 21 days of larval age.

No appreciable differences were found between the two groups in pupal weight, developmental time, and the percentage of infested larvae that survived to the pupal stage. However, the group reared without a diet change yielded fewer adults, and the majority of the surviving moths failed to mate and reproduce. The group provided with fresh diet at 21 days showed good survival and reproductive performance.

The overall results of this test suggested that unless the diet is significantly improved, it is necessary to change the diet at least once to insure high survival of reproductively viable adults.

Recently some important changes were made with regard to the diet and its mode of preparation (and the quality of wild stock) that collectively have resulted in successful rearing of colonized and wild stock without a change of diet. The changes included doubling the vitamin mix to provide a total of 10 g per liter of diet. This step provides additional compensation against vitamin loss in the diet during the prolonged larval feeding period and additional food preservative action from the antioxidants, ascorbic acid, and alpha-tocopherol that is included in the vitamin mix. Also, all diet ingredients were boiled during processing to inactivate autolytic enzymes and microbial contaminants that promote food spoilage.

Although the tests involving the new procedures are not yet completed, it can be stated that the duration of larval feeding has been significantly decreased. Also, survival and developmental performance of insects reared without a diet change are comparable to that obtained earlier with a change of food at 21 days.

Table 6.5-9.—*Effect of diet[1] change on development of the gypsy moth (Pennsylvania wild strain)*

	No change of diet	Diet changed at day 21
Weight (grams)		
Pupal weight of males	0.50 (.08)[2]	0.52 (.07)
Pupal weight of females	1.94 (.25)	2.14 (.54)
Developmental time		
Days to adult, males	48.3 (2.5)	46.4 (1.8)
Days to adult, females	51.2 (3.8)	49.0 (3.1)
Survival (percent)		
Pupal yield	88.7	95
Adult yield	71.0	91
Normal males	59	95
Normal females	0	91
Reproduction		
Reproductive females (percent)	40	90
Eggs laid per female	329	840 (290)

[1]Reared on high wheat germ diet at eight larvae per ME-6R container.
[2]Data represent $\bar{x}$ values, with standard deviations in parentheses.

Temperature Effects on Diet Quality

Tests were conducted to determine if the temperature at which diet ingredients were subjected during processing affect the quality or nutritive value of the diet. Thus, in one test, high wheat germ diet was prepared in a blender and ingredients were added to the water/agar suspension at 100°, 90°, 80°, and 70° C. Another batch of diet was autoclaved.

In another test designed to simulate a mass-rearing situation where large volume kettles would be employed, diets were cooked at 80° C for 30, 60, 90, and 120 minutes before dispensing.

The results of these tests indicated that the overall quality of the diet was improved by a brief exposure to 100° C, prolonged exposure to 80° C, or by autoclaving. The development time for the gypsy moth was noticeably faster on diets that had been cooked for longer periods (1 hour or more.) Also, the incidence of egg hatch was significantly higher in treatments involving exposure of the diet ingredients to 100° C (table 6.5–10) and to autoclaving.

Undoubtedly the benefits derived from cooking the diet at high temperatures and for prolonged periods are significant. The high temperatures during processing are necessary to inactivate food spoiling enzymes and microbes, and there may be additional benefits such as an increase in the gelling properties or water binding characteristics of the diet.

As pointed out by Singh (1977), the conventional methods of preparing diet, by cooling ingredients to 70° C before adding vitamins, may not be necessary. During these program studies no adverse effects on development of the gypsy moth were found when vitamins or vitamin sources were boiled for 5 minutes versus incorporation after cooling the diet to 70° C.

Further studies are needed to determine the optimum temperature conditions for treating and holding the diet during processing. Much of the variation observed in development and particularly in egg hatch may have resulted from temperatures to which dietary ingredients are subjected during processing. Too frequently the diet was probably processed at temperatures that were too low rather than too high.

To insure production of diet of uniform quality, it is advisable to install an automatic process control system so that each batch prepared is processed according to a preset time and temperature schedule and monitored by temperature chart recorders.

Agar Substitutes

Because agar accounts for 30-50 percent of the total dietary cost, considerable effort has been made to reduce the agar content or to find suitable alternative gelling agents. One advantage of the high wheat germ

Table 6.5–10.—Effects of temperature during diet processing on development of the gypsy moth[1] (high wheat germ diet)

Temperature[2] (° C)	x̄ Developmental time (days)[3]		Percent adult yield[4]	Egg-mass weight (mgs)	Percent hatch[5]
	Males	Females			
70	45	47	90	621	42[a]
80	46	49	96	696	35[a]
90	47	47	92	665	27[a]
100	48	46	95	808	70[b]

[1]Stroudsburg, Pa., wild strain; aggregately reared at 10 larvae per ME-6R cup, with a diet change at 14 and 28 days of larval age.

[2]Temperature at which nutrients and vitamins were added to water/agar mix.

[3]From larval hatch to adult.

[4]Adults recovered from initial larvae placed on diet (N=120 per treatment).

[5]Hatch data followed by same letter are not significantly different.

diet is that it can be formulated with 25 percent less agar than used in the previous diets and still produce a strong gel which results in a considerable reduction in cost.

Preliminary experiments in which several different gelling agents were evaluated indicated that Gelcarin® HWG (a hydrophilic colloid derived from red seaweed marketed by Marine Colloids, Springfield, N.J.) may provide a suitable alternative to agar.

A series of experiments was carried out with formulations that contained Gelcarin® HWG at concentrations ranging from 0.75 to 2 percent. In some treatments, locust-bean gum was added to the HWG at 0.5 to 1 percent. These formulations were compared with the standard high wheat germ diet with 1.5 percent agar.

The results showed that the diets formulated with HWG produced gels that were as good or better than those produced with agar. Growth and development of larvae reared on diets with HWG vs. agar were comparable. Concentrations of HWG above 1 percent or the addition of locust-bean gum did not significantly improve the gelling properties of the diet or growth response of the larvae. The cost of the high wheat germ diet with 1 percent HWG is about $0.24 per liter. The physicochemical properties of HWG, however, are different from that of agar, and HWG gels at a much higher temperature. When using HWG, it is advisable to hold the processed diet at a minimum of 80° C, with frequent stirring during holding and dispensing and minimum exposure of diet to the air. Moreover, HWG should be dissolved in very hot or boiling water, and nutrients or dry ingredients should be added while the water-HWG is above 80° C.

Effect of pH

An experiment was conducted to determine the optimal pH of the diet for insect development and for preventing microbial contamination. The test involved the high wheat germ/casein and high wheat germ/soy protein formulations with and without microbial inhibitors (methyl paraben and sorbic acid). The purpose of the test was to determine if the pH of a diet affects development and incidence of microbial contamination. The diet was prepared in liter batches in a blender, and the pH adjusted by addition of concentrated hydrochloric acid or 4 molar potassium hydroxide. The pH was determined on hot, blended diet using a Beckman SS-3 pH meter. Previous studies showed little difference in pH readings obtained from dispersed ingredients in cold water and hot (90° C) blended diet. Diet was poured into 180-ml (XE-6) plastic cups, and 10 newly hatched larvae were placed in each cup and reared through larval development without a diet change. Containers were inspected daily for microbial contamination. Larvae from the various treatments were sexed and weighed at 27 days after initial placement on diet. The pH of the different treatments ranged from 3 to 8.

Elimination of the antimicrobial agents from the diet resulted in contamination of all diets with a pH of 4 or above. Larvae grew poorly at pH levels below 4. Antimicrobial agents were ineffective in suppressing contamination at a pH in excess of 6.5. Contamination was greater on the wheat germ/casein than on the wheat germ/soy protein. Growth of the larvae was retarded at pH 7 or above in both diets. It is concluded that a pH ranging from 4.5 to 6.5 is most satisfactory for rearing the gypsy moth, with contamination problems being minimal toward the more acid end of this pH range. However, since the natural pH of both diets falls within a range of 5.3–5.8, no considerable advantage can be gained by either increasing or decreasing the pH level.

Antimicrobial Agents

Several antimicrobial agents were evaluated for relative effectiveness in suppressing contamination of the diet. An effort was made to find a concentration of a single antimicrobial agent that would prevent any microbial contamination of the diet. The test diet was the high wheat germ diet; the wheat germ used was old and heavily loaded with microbial contaminants. Diet was prepared in the conventional manner with a blender and 10 larvae were reared per cup. After 7 days at 25° C, the surface of the diet was examined for microbial growth. Each treatment involved 30 cups.

Results (table 6.5-11) showed that sorbic acid, methyl paraben, and 10 percent formalin were 100 percent effective in preventing contamination when used at a concentration of 0.4 percent. Even at 0.2 percent, sorbic acid and formalin (10 percent) were about 97 percent effective. These compounds will be used singly in further tests at 0.2, 0.3, and 0.4 percent to determine effects on development and reproduction of the insect.

Rearing Containers

A system of rearing the gypsy moth was devised by APHIS investigators at Otis AFB that involved the use of heavy-duty paper Dixie® cups (2186SE) (Secrest and Collier 1967). Larvae previously reared in petri dishes to the second instar were transferred into the Dixie® cups. Fifteen larvae were placed in each cup along with one or more plastic cream cups, each containing 30 ml of diet. Fresh diet was provided by subsequently replacing the old cream cups with new ones containing fresh diet. Such rearing containers with certain modifications have been used for larger

Table 6.5-11.— *Relative effectiveness of various antimicrobial agents in preventing contamination of hi-wheat germ diet for rearing gypsy moths.*

Antimicrobial agents	Percent	Percent of rearing cups without contamination
Sorbic acid	0.4	100
Methyl paraben	.4	100
Formalin (10%)	.4	100
Sorbic Acid	.2	96.7
Formalin (10%)	.2	96.7
Potassium sorbate	.4	93.3
Glacial acetic acid (10%)	.4	90.0
Sodium propionate	.4	86.7
Sodium benzoate	.4	83.3
Formalin (10%)	.1	83.3
Glacial acetic acid (10%)	.2	80.0
Sodium benzoate	.2	73.3
Calcium propionate	.4	70.0
Sodium benzoate	.1	50.0
Sorbic acid	.1	33.3
Formalin (10%)	.05	30.0
Potassium sorbate	.2	26.7

Containers examined for contamination at 1 week after larvae were placed on diet.

scale rearing for several years. However, this system was unsuitable for efficient mass rearing primarily because insects could not be easily reared from eggs or newly hatched larvae to pupation in such containers. Newly hatched larvae placed in the containers wandered excessively resulting in poor establishment and slow growth in the early instars.

The ideal container for rearing the gypsy moth should be inexpensive and suitable for holding the insect in close proximity to diet from the infested egg to the pupal or adult stage. Thus the container should be small enough to prevent excessive wandering by newly hatched larvae, yet large enough to permit unrestrained development and pupation of several larvae reared in aggregation. Ventilation should be adequate to meet the maximum respiratory demands of the insect without promoting excessive drying of the diet.

In reality, once a prospective rearing container has been selected, a favorable and delicately balanced microclimate must be obtained within the container by adjustment of the ambient relative humidity, air flow, volume of diet, and insect population density.

Over the past few years, several types of containers for individual and aggregate rearing were evaluated. These included several types of polyethylene, styrene, and metal trays, various crispers and food containers, paper and aluminum hexcels, plastic cell-tray units, and various paper and plastic cups with paper or polyethylene lids.

The best success was obtained with plastic food cups fitted with paper (cardboard) lids. (Unfortunately, the range of sizes of food cups that are commercially available and permit the use of paper lids is limited.) The paper lids permitted adequate ventilation and served as a favorable substrate for attachment of the larvae during molting and subsequent pupation. Plastic surfaces were generally unsuitable.

Of several different plastic food cups tested, 180-ml polyethylene cups (XE-6 and ME-6R, fig. 6.5-1, table 6.5-3) were preferred and adopted for subsequent routine testing and evaluation.

A test was conducted to determine the optimal number of insects per container. Preliminary observations had shown that when more than 10

insects were reared to pupation per container, growth and survival were unsatisfactory. The container (XE-6) used in this test had a capacity of 180 ml and was less expensive than the ME-6R container.

Larvae were reared at 4, 6, 8, and 10 per cup; each cup contained 80 ml of diet. Results (table 6.5-12) showed no difference among treatments in the developmental rate, but the percentage reaching the pupal stage was lower in treatments involving 8 and 10 larvae per container. Also, pupal weights were higher in the treatment with 4 larvae per container. There was a decrease in pupal weight of the males as the population density increased, although this was not strictly true for the females in that no difference was seen in the treatments involving 6, 8, or 10 larvae per container. It must be emphasized that there was no considerable competition for available food, but there was competition for space for molting and pupation. Thus in the treatments involving higher densities, some pupae were malformed. Also, differences in sex ratios in the different treatments may account for the lack of a linear relationship between density and female pupal weight. The main point of emphasis is that the containers were suitable for aggregate rearing of eight larvae per container at low cost—approximately $2.50–$3.00 per 1,000 larvae.

Prospects

The prospects for making further reductions in the cost of containerization is encouraging. Containers that are 50 percent larger in diameter than those currently used could accomodate twice as many larvae with a considerable reduction in cost per insect. However, such containers may have to be custom made, as a commercially available source is not known. Alternatively, it has been estimated that by slightly altering the design of the containers used at present to permit heat sealing with paper lidding, the cost could be reduced by 30–40 percent.

Another attractive possibility that needs further exploration is a reusable container system similar to the cell-tray sandwich reported by Raulston and Lingren (1972).

Large-Scale Production Procedures

As a result of the mass-rearing research effort, several constructive changes in the large-scale production of gypsy moths have been made during the last 2 years. For example, the colonies and insects produced for various program needs are now reared either on the modified hornworm or the lower cost high wheat germ diet. Also, the ME-6R or XE-6 containers have largely replaced the petri dishes and Dixie® containers previously used. Labor associated with diet preparation or handling of the insects has been reduced or omitted. Problems with microbial contamination of the diet or with virus disease have been virtually eliminated.

Because many of these procedures were only recently implemented and are likely to be replaced by more efficient procedures shortly, only a brief summary is given here to indicate the status of the present system. These procedures, with slight variations, were used to produce over 10,000 insects per

Table 6.5-12.—*Effects of density on development of gypsy moths reared in plastic cups*[1]

Number infested	Number per container	Days to pupation[2]		Percent pupal yield	Pupal weight (g)[2]	
		Males	Females		Males	Females
40	4	31.7(1.4)	34.3(3.0)	100.0	0.70(.08)	2.4(.41)
48	6	31.5(1.6)	34.7(3.1)	98.0	.67(.10)	1.9(.37)
80	8	32.1(1.4)	35.1(3.7)	88.0	.63(.08)	2.1(.31)
100	10	31.7(1.2)	35.0(2.8)	84.0	.55(.08)	1.9(.34)

[1]XE-6 polyethylene (180-ml capacity)
[2]Figures are mean values with standard deviations in parentheses; pupal weights based on $N = 10$ and 10 per treatment.

day. Although most of the operations are handled by manual labor, the rearing system is still reasonably efficient. The major labor-intensive steps or operations involved in rearing are shown in table 6.5-13.

Preparation and Handling of Diet

Diet ingredients (for either the modified hornworm or high wheat germ) is weighed out into plastic bags in 20 or 40 l batches and stored in a freezer until needed. Diet is prepared in a steam jacketed kettle (40 l capacity) and mixed with a 2 HP Speco® mixer-homogenizer (fig. 6.5-3). After mixing for 5 minutes, the diet is dispensed into rearing containers with a Filamatic® piston-filling machine.

Trays for holding containers of freshly dispensed diet are placed on carts located next to the dispensing equipment. After the diet is cooled down, the carts are transferred to an adjacent room where immature stages (larvae and pupae) are handled.

Handling of Insect Stages

Newly hatched larvae are brushed on to the diet at the rate of 15 per ME-6R cup. However, if larvae are to be reared to pupation without change in diet, only 8

Table 6.5-13.—*Operations and worker-hours involved in rearing and handling gypsy moths*

Operations	Worker-hours per 10,000 insects
1. Diet-weighing, processing, and dispensing	6
2. Infesting larvae	10
3. Larval transfer to fresh diet	10
4. Sexing and transfer to adult emergence containers	30
5. Mating of adults	.1
6. Harvesting eggs	.1
7. Eggs transferred to chilling	—
8. Chilled eggs (clean up and disinfection)	.2
Total	60

¹Operations 1, 2, and 4 may be mechanized, resulting in a 75% decrease in cost of labor. Operation 3 may be omitted altogether. Thus, worker-hours per 10,000 insects may be reduced from 60 to 12–15. Operations 5–8 involve time in handling 50–100 adults for colony production to provide minimum of 10,000 newly hatched larvae per day.

or 10 larvae are placed in each cup. The cups are then sealed with paper lids.

After placement on diet, larvae are transferred to the main rearing room and held at 25° C and 60 percent humidity. After 21 days, the larvae were transferred to 500-ml cups with one or two small 52.5-ml cups of fresh diet.

After pupation is completed, pupae are harvested, sexed, and placed in 500-ml cups at 25 females and 50 males per cup. Just prior to adult emergence, the pupae are transferred to the adult holding room. Upon emergence, each day about 150 pairs of moths are placed in heavy-duty paper containers (3.8 l) at 25 pairs per container (fig. 6.5-4, *A*). The male moths are cooled in a refrigerator at 10° C for several minutes before handling to halt flight activity. After 3–4 days the moths are vacuumed from the containers, and the egg masses are harvested by scraping them from removable paper used to line the inside of the container. The egg masses are held at 25° C for 28 days, to permit embryonation and entry into diapause, and then transferred to a refrigerated chamber (fig. 6.5-4, *B*) for termination of diapause.

After chilling for 4–5 months at 6° C, the egg masses are removed, surface disinfected with Formalin (10 percent), and placed in petri dishes (3–5 masses per dish) for hatching. The egg masses are left intact during disinfection and larval hatching because it was found that removal of the hairs frequently reduces the incidence of hatch and involves extra labor.

Sterile Male and Virus Production

An alternative rearing system was devised and implemented for the simultaneous production of males (for sterile-male field tests) and females for virus production tests. Early-stage larvae were reared as usual: 15 larvae were initially placed into each cup and held for 21 days. At 21 days, when sexual dimorphism was evident, the larvae were sexed and transferred to XE–6 containers with fresh diet. The male larvae were reared to pupation at 15 per cup and harvested for subsequent sterile male studies. The 21 day-old females, which obviously could not be used for sterile

Figure 6.5-3.—*Steam-jacketed kettle, mixer-homogenizer, and dispensing equipment used in the preparation of gypsy moth diet.*

male work, were reared at five per cup for production of polyhedrosis virus. Thus the simultaneous use of insects for sterile male and virus production permits utilization of the otherwise unneeded females.

Operations and Labor Requirements

Table 6.5–13 summarizes the major operations and labor involved in the previously described rearing system. The labor-intensive operations are infesting of larvae, transfer to fresh diet, and pupal harvest and sexing. By using technology described elsewhere in this chapter, it should be possible to automate such labor intensive operations. If automation can be implemented for infesting of eggs and pupal harvest (and sexing), along with omission of the transfer of larvae to fresh diet, only two workers (instead of the seven or eight currently needed) would be required to rear 10,000 insects per day.

Cost Considerations

Obviously, the cost of rearing the gypsy moth varies, depending upon whether the insects are

Figure 6.5-4.—*Handling of adults and egg masses for colony maintenance: A, Mating of adults in 3.8-1 paper containers; B, containers of egg masses are held on conveyor in refrigerated chamber for 180 days to terminate diapause and synchronize egg hatch.*

required for virus or parasite production, sterile male programs, perpetuation of laboratory colonies, or other purposes. Thus, each requirement is accompanied by different product specifications that also have different inherent cost factors. For example, to produce the polyhedrosis virus, it is only necessary to rear the host larvae, perhaps to the end of the larval feeding period. The actual rearing costs for virus production can therefore be much less than for sterile-male production, which involves not only rearing of the larval stages but also additional labor for the harvesting and sexing of the pupae. Also, 2,000 larvae must be reared to obtain 1,000 insects of the desired male sex. Additional cost factors may be involved to insure that the sterile males show the desired degree of performance in the field.

The highest cost per insect may be incurred in the maintenance of laboratory colonies, since special care must be taken to insure not only that such stock is free of disease but also that appropriate genetic (or behavioral) traits and a high potential for population increase are maintained. However, to maintain a primary colony of gypsy moths that will yield 100,000

newly hatched larvae per day, it is necessary to rear only about 250 females or 500 insects per day.

The present costs (diet, containers, and labor) for rearing gypsy moths for virus production, sterile males, and colony maintenance are estimated at $12, $18, and $27 per thousand insects, respectively. It is expected that these costs can be further reduced by one-third to one-half if envisioned labor-saving techniques are implemented.

Quality Control

An up-to-date assessment of quality control and guidelines for quality monitoring of mass reared insects have been dealt with by Chambers (1977) and Boller and Chambers (1977) and need not be reiterated here. The concepts presented and procedures developed by these workers will have a dramatic impact on future programs involving mass-reared insects. The proper development and application of such concepts should insure production of insects that are reasonably efficacious.

Currently, procedures are being developed for assessing the quality of mass-reared gypsy moths for various program needs. Performance data are being gathered on healthy wild populations to establish standards with which measurements of colonized strains can be compared. The following is a brief description of this aspect of the research program.

Biotic Potential

The ability of the populations to thrive under colonized conditions in the laboratory is being assessed by determining the biotic potential or intrinsic capacity for increase. To determine the biotic potential, it is necessary to measure fecundity—that is, incidence of mating, eggs per female and hatch, developmental time, and survival (adults recovered per number placed on diet). Use of the intrinsic capacity for increase as a measure of performance of laboratory-reared gypsy moths was explained earlier (see section on Test Insect Populations).

Environment

Those components of the rearing environment that affect the biotic potential are being studied to determine the optimum environment for mass rearing. The environmental factors under assessment include temperature, humidity, diet, light, handling and containment of developmental stages, and incidence of viable and nonviable contaminants. Once these factors are evaluated and the optimal rearing environment established, a standarized set of environmental conditions for rearing will be prescribed as well as methods by which each of these factors can be routinely monitored to assure that the quality control of the rearing environment is maintained within an acceptable range of tolerance.

Efficacy

The final phase of the quality-control research is that of determining efficacy of the mass-reared insects to achieve program objectives. The programs involved include virus production, parasite rearing,

pheromone evaluation, and evaluation of the sterile-male technique.

It is intended that suitable methods will be devised to measure efficacy of the mass-reared stock for each of these programs. The appropriate quality control measurements will be made and standards established by SEA, APHIS, and Forest Service personnel. As required, modifications will be made with regard to the strain of insect reared or the rearing environment to meet quality specifications.

Monitoring Colony Performance

A system has been implemented for routinely monitoring the developmental and reproductive performance of the main colony. Key factors that need to be measured and respective data obtained are summarized in table 6.5-14 and indicate the growth and development rate as well as the intrinsic rate of increase of the colony. Overall, the performance of the colony seems satisfactory, but the rate of increase value Ri is far less than the known potential for this strain. For example, in recent experiments it has been possible to obtain potential Ri values greater than 1,000×. It is therefore evident that methods for

Table 6.5-14.—*Performance of a long-term colonized(NJF_{16}) strain of gypsy moths under mass-rearing conditions*

	Mean values, with ranges
Growth and development	
Pupae weight g, males	0.63 g (0.60–0.70)
Pupae weight g, females	2.1 g (2.0–2.2)
Dt_{50}[1] (days) to adult, males	41 (40–43)
Dt_{50}[1] (days) to adult, females	42 (41–44)
Survival and reproduction	
Adult female survivors	83%
Reproducing females	90%
Reproducing females per initial N[2]	74%
Eggs per female	733
Hatch	74% (40–94)
Ri[3]	406×

[1]Dt_{50}= time in days required for 50 percent of the test population to develop to the adult stage.
[2]Percentage of females (N) initially placed on diet.
[3]Ri= rate of population increase per generation per female.

maintaining the brood colonies can be greatly improved. Efforts are now being made to improve the survival of reproductive females, the numbers of eggs per female, and particularly the incidence and uniformity of egg hatch. The implementation of improvements in processing of the diet and adjustments in density of larvae per container will undoubtedly increase the colony performance. Optimization and standardization of diet processing and environmental conditions for holding of the eggs prior to and during diapause should result in a consistent and improved egg hatch.

Development and Evaluation of a Rearing Facility

A Prototype Facility

A prototype or pilot-scale mass-rearing facility for the gypsy moth was needed to gain more precise information on spatial needs, requirements for environmental control and special equipment for worker protection, methods and equipment for increasing production efficiency, and problems related to contamination and sanitation.

The facility would permit testing and evaluation of certain automated procedures developed by earlier investigators for mass-rearing other insect pests. For example, the diet on which some insects are reared is passed through a flash sterilizer before it is automatically dispensed (Griffin and Lindig 1974). A form, fill, and seal machine has been used to form plastic containers that are then filled with sterile diet, implanted with insect eggs, and sealed, all in one continuous operation (Harrell et al. 1973, 1977).

Various methods for machine dispensing of eggs on artificial diet have been developed (Gant 1966, Harrell et al. 1970, 1974). Methods for machine harvesting of pupae have also been developed (Harrell et al. 1969, 1974).

A major problem in many mass-rearing facilities is the presence of scales or hairs that become dislodged from the insect, particularly moths, and that cause skin or respiratory problems in susceptible workers.

Cyclone separators were successfully used as a means of removing such contaminants from mass-rearing facilities (Harrell and Perkins 1971, Ridgway and Billingsley 1977).

Design Criteria

A prototype mass-rearing facility was designed to provide areas to prepare experimental diets and evaluate diet processing equipment, for storage and weighing of diet ingredients and for infesting and handling immature stages of gypsy moth. Separate rooms were designed for holding the immatures and adults in which temperature, humidity, air flow, and clean air filters could be easily changed to provide an optimal environment. Also the rooms had to be large enough to permit testing of mechanization and to simulate mass-rearing. An isolated area was also needed for virus production.

Basic Layout

Three surplus mobile homes obtained from Housing and Urban Development were used to construct the prototype facility (fig. 6.5-5). One unit (3.6×15 m) was used for weighing and storage of diet ingredients (fig. 6.5-5, A), diet preparation (fig. 6.5-5, B), and establishment of insects on diet (fig. 6.5-5, C). The other unit of similar dimensions was modified and used largely as a room for rearing insects on experimental diets (fig. 6.5-5, D). The larger unit (3.6×18 m) (fig. 6.5-5, G) was used for production and colony maintenance. An adult holding room (fig. 6.5-5, E) and another room (fig. 6.5-5, I) that connected the kitchen with the adult holding and mass-rearing rooms made up the remainder of the facility.

To convert the mobile homes into a suitable facility for rearing gypsy moths, it was necessary to remove the old floor covering and the interior walls to facilitate cleaning. In two of the units the interior walls and ceiling were removed and replaced with vinyl covered gypsum board (Homosote). In the mass-rearing unit, only the floor covering was replaced.

(G) Colony and production

(H) Work area

S

(A) Diet storage

To Lav.

(I)

(F)

(E) Adult Handling

(C) Diet Preparation and infest.

(D) Prototype Rearing

S

(B)

1/8″ = 1′
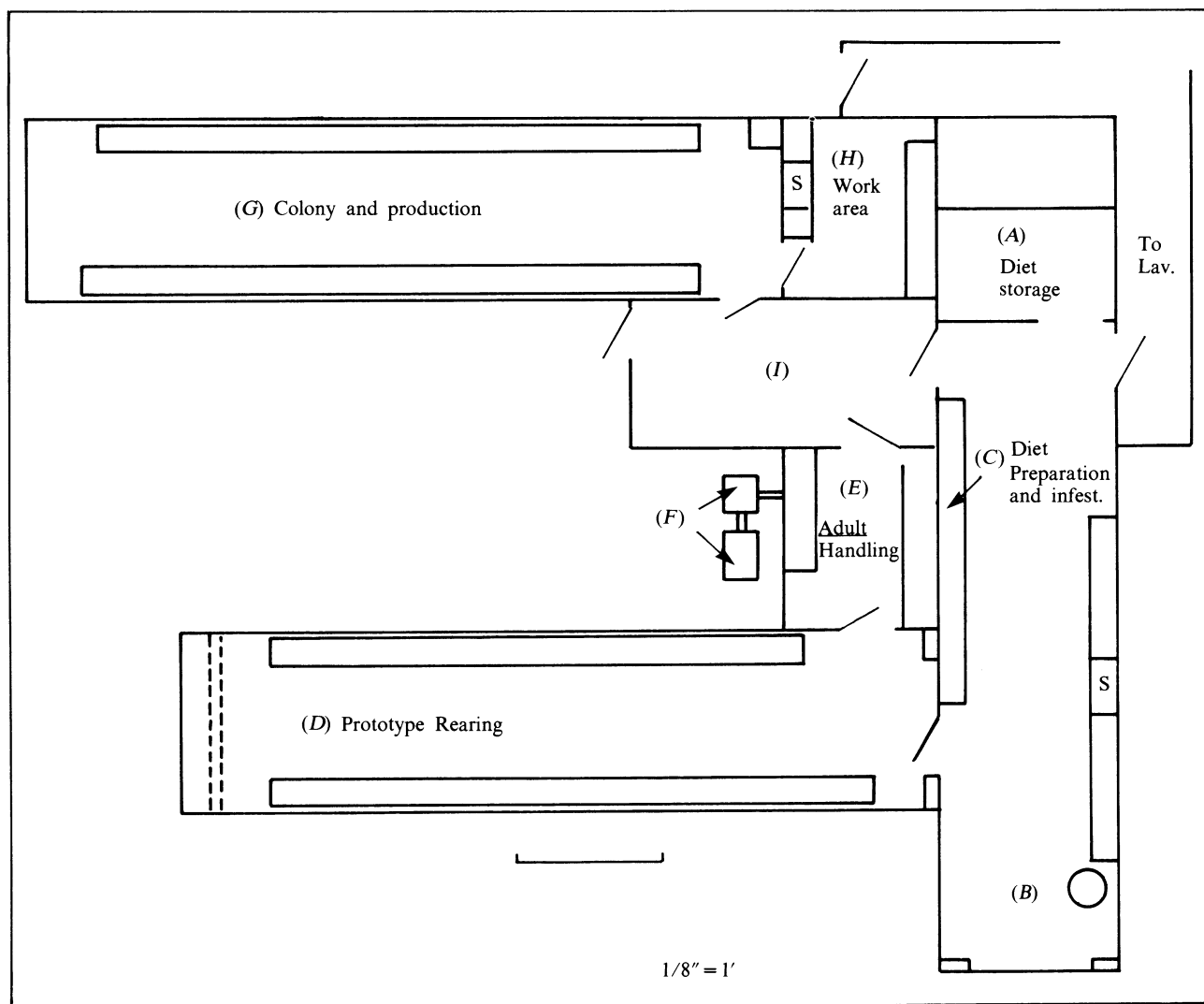
Figure 6.5-5.—*General floor plan of a prototype gypsy moth rearing facility constructed from modified mobile homes:* A, *Diet storage and weighing;* B, *diet preparation area;* C, *larval infesting area;* D, *experimental or prototype rearing room;* E, *adult holding and handling;* F, *cyclone separator and dust collector for the adult room;* G, *colony rearing and production;* H, *larval transfer and pupal harvest;* I, *common room connecting colony rearing and production with the diet preparation infest and adult rooms.*

Air Flow Systems

Each mobile-home unit and the adult room were provided with separate air handling and conditioning equipment. The diet preparation and the prototype rearing units were equipped with a horizontal airflow system designed to flow lengthwise from one end of the room to the other. In the prototype unit, the maximum airflow was about 30 m per minute (that is, two changes per minute), whereas in the diet-preparation unit the flow rate permitted one air change per 2 minutes. Also both units were equipped with HEPA filters (95 percent) to provide clean air. In the prototype unit, conditioned air was passed through nine ($60 \times 60 \times 30$ cm) HEPA filters (fig. 6.5–6) situated in one end of the room and exhausted through six ($60 \times 60 \times 5$ cm) prefilters at the other end. The main blower was selected to deliver 238 m^3 per minute at 3.75 cm water. The arrangement of the air-handling and air-conditioning system for the horizontal laminar flow unit is shown in figure 6.5–7. About one-sixth (42 m^3 per minute) of the total airflow was diverted through the air-conditioning system.

To provide a comparison with the horizontal air flow system, the colony and production unit was provided with a vertical airflow system (fig. 6.5–8). The rate of airflow was set at one change per 2.5
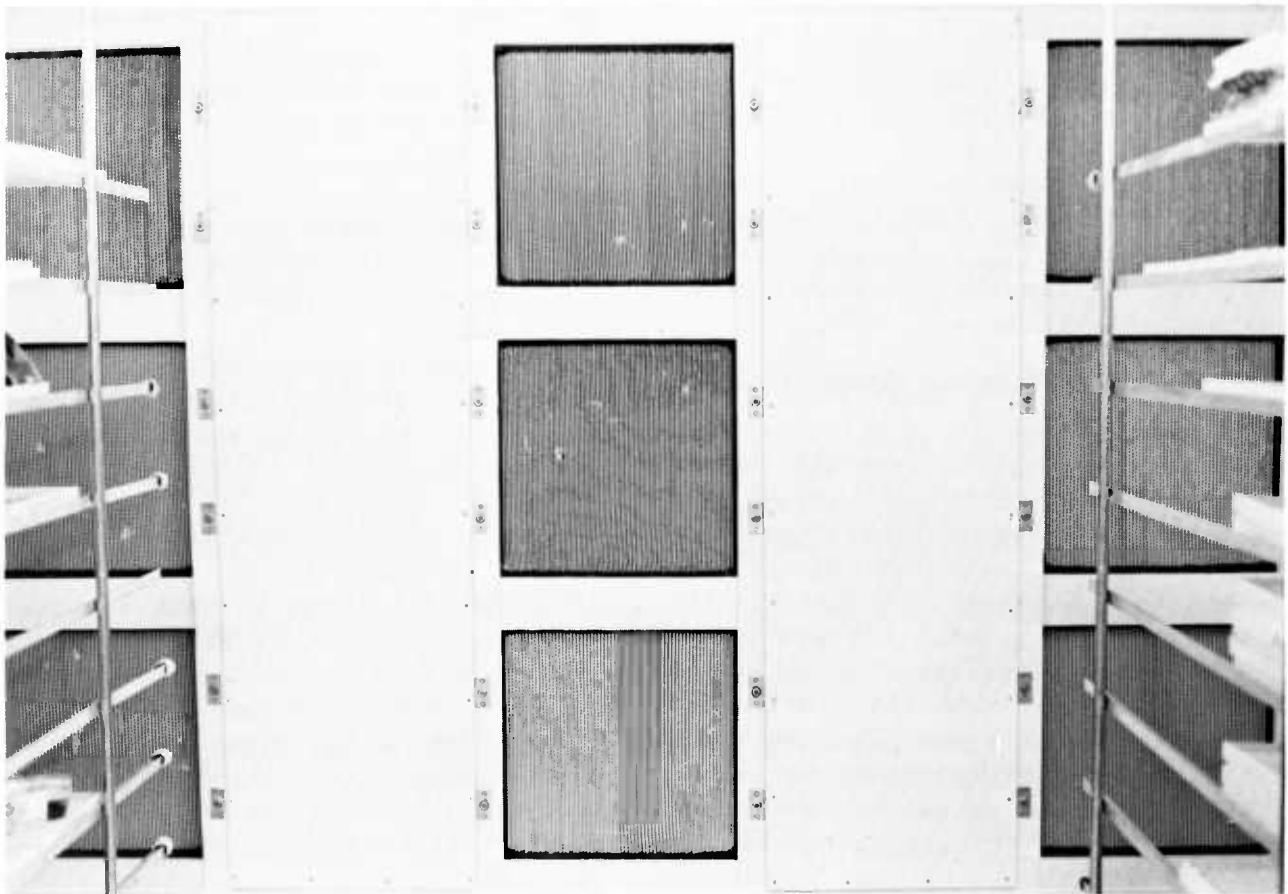
Figure 6.5–6.—*Arrangement of HEPA filters in one end of the experimental prototype rearing room.*
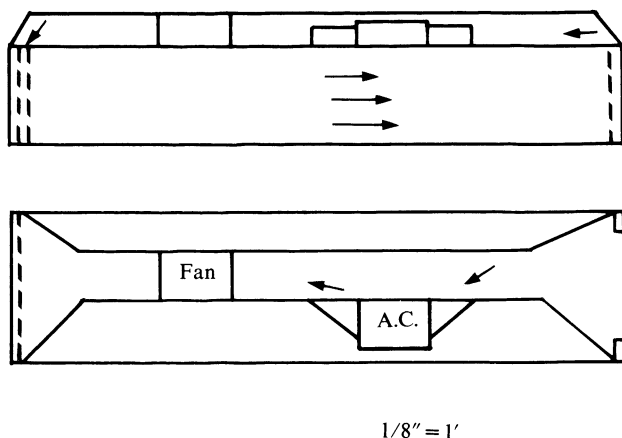
Figure 6.5-7.—*Schematic of the air-conditioning and distribution system in prototype rearing room.*

minutes. The HEPA filter and prefilters were installed in the main duct located on top of the mobile home unit.

The primary reason for installing HEPA filters in all units of the rearing facility was to provide continuous cleaning of the air to remove insect hairs and scales. They were also used for reducing the incidence of airborne microbial contaminants.

Work Stations for Handling Adults

Maximum exposure of workers to insect hairs occur during the process of transferring adults to containers for mating and oviposition and later when they are removed and eggs harvested. To protect the workers when handling the moths and eggs, work stations with high-velocity exhaust ducts were built into the adult holding room. The work stations consist of plexiglass hoods (fig. 6.5-9) that serve to funnel the air through slots in a metal partition located at the rear of the hoods. The design is such that when working close to the slotted partition, the velocity of air (305 m per minute) is sufficient to vacuum intact moths from the holding containers. By moving a short distance away from the slots where the air velocity is much lower, the moths can be handled and only the dislodged hairs or scales are removed.

Larger objects, such as intact moths, are exhausted

through a cyclone separator and deposited in a 210-l drum (fig. 6.5-10, *A*). Smaller particles down to 0.5 μ are removed by a bag filter (fig. 6.5-10, *B*). Smaller particles are removed by an absolute HEPA filter (99.99 percent).

Evaluation of Performance

Conditioning only part of the total circulated air has resulted in very good control of temperature and humidity. A steam generator is used to provide humidity. The airflow in the prototype rearing unit was too high initially; this caused excessive drying of the diet. The flow rate was reduced to 15 m per minute, and satisfactory temperature control (±1° C) was still achieved.

The vertical airflow system in the mass rearing unit also functioned adequately except for occasional periods during the summer when airflow was restricted by holding too many containers of insects in the room.

Some problems occurred with the cyclone separator blower used to circulate the air in the adult holding room. Excessive heat generated by the cyclone fan caused a temperature increase in the room, which required the installation of a supplementary air-conditioner.

After 6 months of operation the fan in the cyclone separator was out of balance because of insect debris sticking to the blades. The metal fan was replaced with a plastic-coated fan and no more trouble has developed.

Recently a portable particle counter (Coulter model 550) was used to determine the efficiency of HEPA filters by measuring the levels of airborne particles in the facility. For comparison, readings were also taken in other locations outside the rearing facility. The kinds of particles are yet to be determined, but the relative amounts indicate the effectiveness of the HEPA filters and airflow design in reducing airborne particles (table 6.5-15). The lowest counts were found in the prototype unit.

The higher counts in the rearing trailer with the vertical airflow system resulted in part from a leaking HEPA filter. The filter was repaired, and the levels of particles have subsequently dropped.

End view

Cross section

HEPA

A C

Fan

Top view
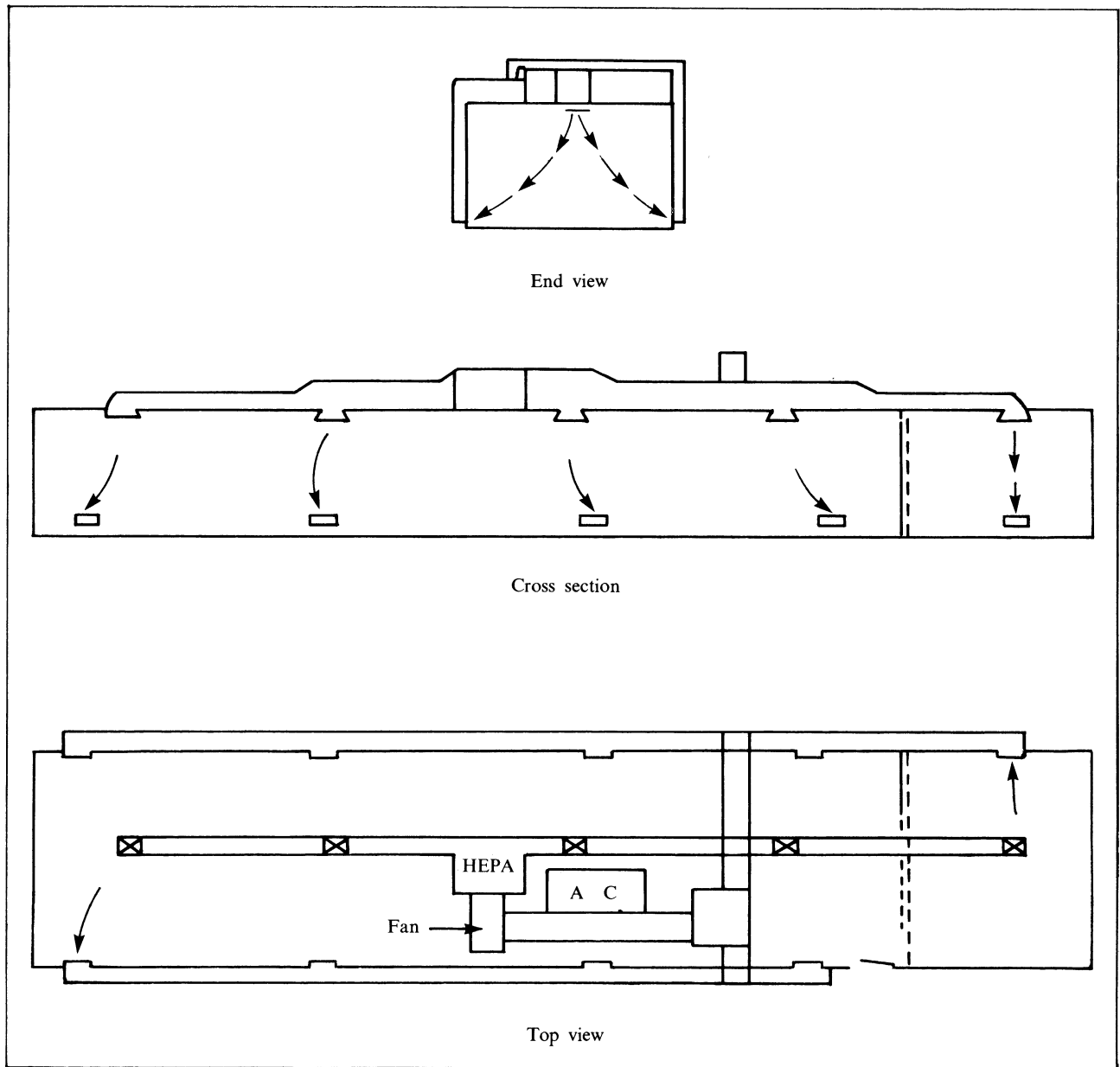
Figure 6.5-8.—*Diagram of air-conditioning and distribution system for the colony and production rearing room, with HEPA filter located in the main duct.*

Figure 6.5-9.—*Special work station for handling adult gypsy moths and harvesting egg masses.*

The high counts of particles in the environmental chamber indicated a need for more rigid sanitation to prevent contamination of the diet when containers are opened. Frequently during the summer months problems occurred with excessive microbial contamination in the chambers.

The particulate counts in the adult holding room were high enough to suggest that some moth scales were not being removed from the room. Measurements made before, during, and after dumping large quantities of moths into the exhaust hood did not result in an increase in particle counts in the air returning to the room.

The particle counter is a useful tool for indicating effectiveness of HEPA filters and sanitation measures in rearing facilities. Establishment of a correlation between levels of airborne particles and probability of microbial contamination is being investigated. The more important use may be in monitoring for levels of insect scales and hairs in areas where workers are exposed.

Equipment

Diet Preparation and Dispensing

Previously used equipment for preparing and dispensing diet at the APHIS mass rearing facility involved a 112-l steam-jacketed kettle, a transfer pump, and an automatic filling machine for

Figure 6.5–10.—*Industrial dust-collecting equipment used for removal of moths and insect debris from the adult holding room:* A, *Cyclone separator;* B, *bag filter unit.*

Table 6.5–15.—*Levels of particles in air sampled in various locations within the laboratory and rearing facility*

Locations	$\bar{x}$ particles/m³ air per minute	
	0.5 to 5 μ	+5 μ
Library—conference room	1,638	7.6
Environmental chamber without HEPA filter	1,783	1.6
Environmental chamber (with portable HEPA filter)	557	1.3
Rearing trailer, colony (vertical airflow, HEPA filter, 95%)	15	1.2
Rearing trailer, prototype (horizontal laminar flow, HEPA filter, 95%)	3	.06
Adult moth room	38	.74
Adult moth room (during handling of adult moths and egg masses)	37	—

dispensing diet into 30-ml creamers and a capper to automatically place plastic closures on the cup. The cups were filled with diet, capped, and placed in a refrigerated room until needed (Tardif 1975).

There were several disadvantages inherent with this type of equipment. The kettle was designed mainly for bakery use and, therefore, had limited mixing capacity (low rpm stirrer and scraper). Batch sizes under 20 l could not be mixed. The filling machine could only handle one container size and had limited flexibility in regard to volume dispensed. Thus when new, lower cost diet and containers were developed, most of the old diet preparation equipment could not be used without considerable modification.

It was envisioned quite early that the type of equipment best suited for processing and handling diet must have several features, including capability of accommodating a wide variety of batch sizes, rapid and thorough mixing of the product, precise control of temperature during processing, and wide flexibility in dispensing the product with regard to rate and volume. Moreover, the equipment should be easy to service, clean, and sanitize and simple to operate. With these criteria in mind, several components were pilot tested. A 40 l steam-jacketed kettle (Hamilton) was used to prepare batch sizes ranging from 10 to 40 l. Water was piped into the kettle at 80° C and heated by steam to boiling within 5–6 minutes. Upon reaching a boil, the steam was automatically cut off and the gelling agents (agar or Gelcarin) were quickly blended in with the aid of a 2 hp Homomixer® (Speco, Inc., Beverly, Mass.) with a variable speed control up to 10,000 rpm. The mixer can be raised or lowered to different depths within the kettle and can be adjusted to attain differential vortex action.

The water/agar mixture was quickly cooled down by flushing out the steam in the jacket and running cold water through the jacket until the desired temperature was reached and then replacing it with steam. The steam held within the jacket permits holding of the diet within the desired temperature range for dispensing. This procedure only requires a few minutes. At 80° C, the dry mix is blended in using

the Homomixer® at high speed. Diet is then dispensed with a piston-action filling machine (Filamatic®) into containers and loaded on carts to cool before introduction of larvae. By using two piston fillers, one worker can fill 40 cups per minute at volumes ranging from 10 to 130 ml per cup. Thus, in 1 hour a worker can fill 2,400 cups with 90 ml of diet, which is sufficient to rear 24,000 gypsy moths. By adding larger pistons and two more filling lines the capacity can be doubled.

The Speco Homomixer® does a thorough job of mixing if permitted to run for several minutes; however, a holding kettle of smaller diameter with a more cylindrical shape would permit the mixer to operate more efficiently. The diameter of the present experimental kettle (50 cm) is about 20 cm too large.

Several units for mixing diet have been tested over the past several months. Of these, LIKWIFIER®, (manufactured by Breddo Food Products, Kansas City, Mo.), was very efficient. The unit is a combination steam-jacketed kettle-mixer. With high-pressure steam (40 psi), the combined time to heat water to boiling, mix in the agar and remaining nutrients, and pump to an adjacent holding kettle for dispensing should require 10-15 minutes. The 68 l unit should process 3 to 4 batches or over 200 l per hour (sufficient diet to rear 20,000 insects).

None of the equipment previously tested or currently used is considered highly satisfactory for diet processing. A kettle-mixer will be tested (vacuum/pressure AGI-Mixer®, Greerco Corp., Hudson, N.H.) in the next few months that will permit rapid mixing and homogenization of diet as well as sterilization and vacuum processing. The mixer is equipped with batch process control and time and temperature recording equipment to provide uniformity of processing. Such a unit may eliminate what is perhaps one of the most troublesome problems in the present mass-rearing system, the variation in day-to-day processing of the diet.

Multistation, Clean-Air Worktable

A clean-air worktable (figs. 6.5-11 and 6.5-12) large enough to accommodate up to ten workers was constructed for handling immature stages of the gypsy moth. Air within the room flows over the work areas and is drawn through prefilters and HEPA filters before reentering the room. Thus, workers can handle the various immature stages without being unduly exposed to the hairs. The airflow rate can be adjusted for either high or low volume. Each work station is also equipped with a hose connected to a central vacuum cleaner for removing cast skins and other insect debris during larval transfer or pupal harvest.

Conveying and Holding of Diet and Insects

Several carts (0.6×1.2×1.8 m) were constructed for holding and moving diet and insects within the rearing facility. Each cart is constructed of aluminum and can hold 10,000 or more insects. Trays for holding containers of insects are constructed from poly-ethylene light-diffusion louvers. The tray units were made in two sizes (60×60 cm and 60×40 cm) and equipped with rollers. The larval, pupal, and adult holding rooms are equipped with several tiers of aluminum tracks or rails that, together with the trays, serve as shelving units. Trays of containers with insects are placed on rails at one end of the room and are sequentially rotated on a daily basis along the railing in an assembly line fashion. Pupae and adults are removed at the end of the line. This rotational procedure ensures developmental uniformity, because all insects are moved through the same environmental conditions. Insects of a given age always occupy the same position along the track; this enables the workers to set up check points along the route at appropriate positions to monitor development. Generally speaking, the procedure allows a more orderly and uniform production schedule.

Egg Storage and Chilling Room

A refrigerated walk-in chamber (3 m×4.6 m) was modified to provide efficient handling and uniform chilling of gypsy moth egg masses (figs. 6.5-4, B, 6.5-13). To accomplish this, each day's production of eggs (after prediapause development) is transferred to

Figure 6.5-11.—*A multistation clean-air work table for handling immature stages of the gypsy moth.*

the cold chamber and placed on a conveyor. Each day the conveyor motor is activated by a timeclock and rotates the eggs to a new position within the chamber. After 180 days, the eggs are advanced to the end of the conveyor and removed each day. Despite some variations in temperature and airflow within the chamber, because of rotation through the chamber during chilling, all eggs experience similar temperature conditions for the same duration of time. The unit is equipped with backup refrigeration that takes over if the main unit malfunctions.

Automation

Of the various operations involved in rearing the gypsy moth (table 6.5-13), processing and dispensing of diet, infesting larvae, and harvesting and sexing of pupae require by far the most labor. Therefore, emphasis has been placed on devising methods for mechanizing these operations.

The recently acquired LIKWIFIER® mixer has significantly reduced the time involved in mixing the diet. The addition of a conveyor system for automated filling of containers with diet and transport to the existing mixing unit will bring about further cost reductions.

The greatest drawbacks to development of an automated system for rearing have been the lack of synchrony of egg hatch and the necessity for changing the diet during larval feeding. Both of these problems will be resolved shortly with further research. Synchrony of hatch is a problem only with the

To vacuum cleaner

Supplies

Fan

Filter
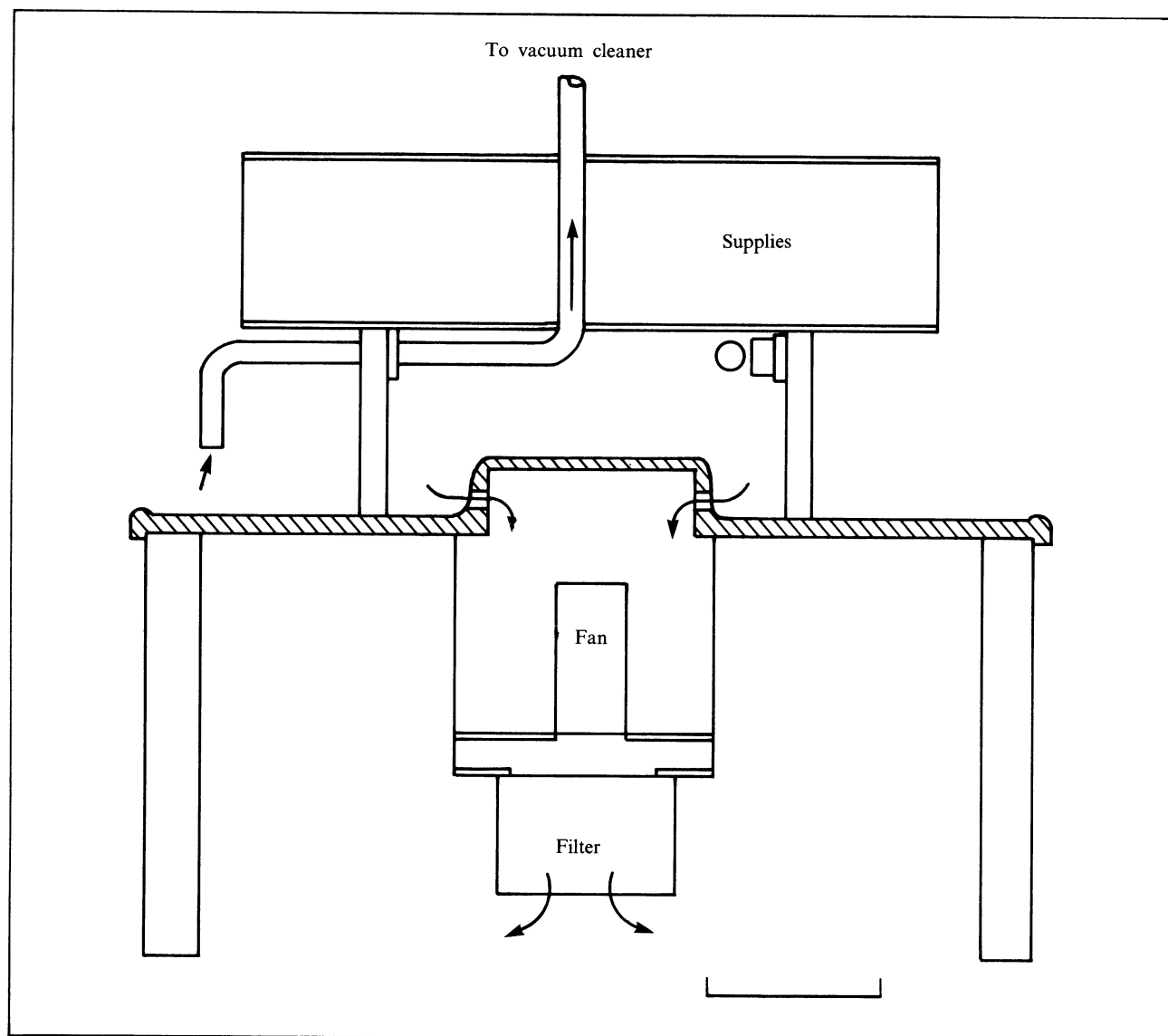
Figure 6.5–12.—*Cross section of a clean-air worktable for handling various stages of the gypsy moth. The unit includes a storage component for temporary holding of rearing containers, a vacuum system to remove cast skins and other insect debris, and a filtering system to remove insect debris.*
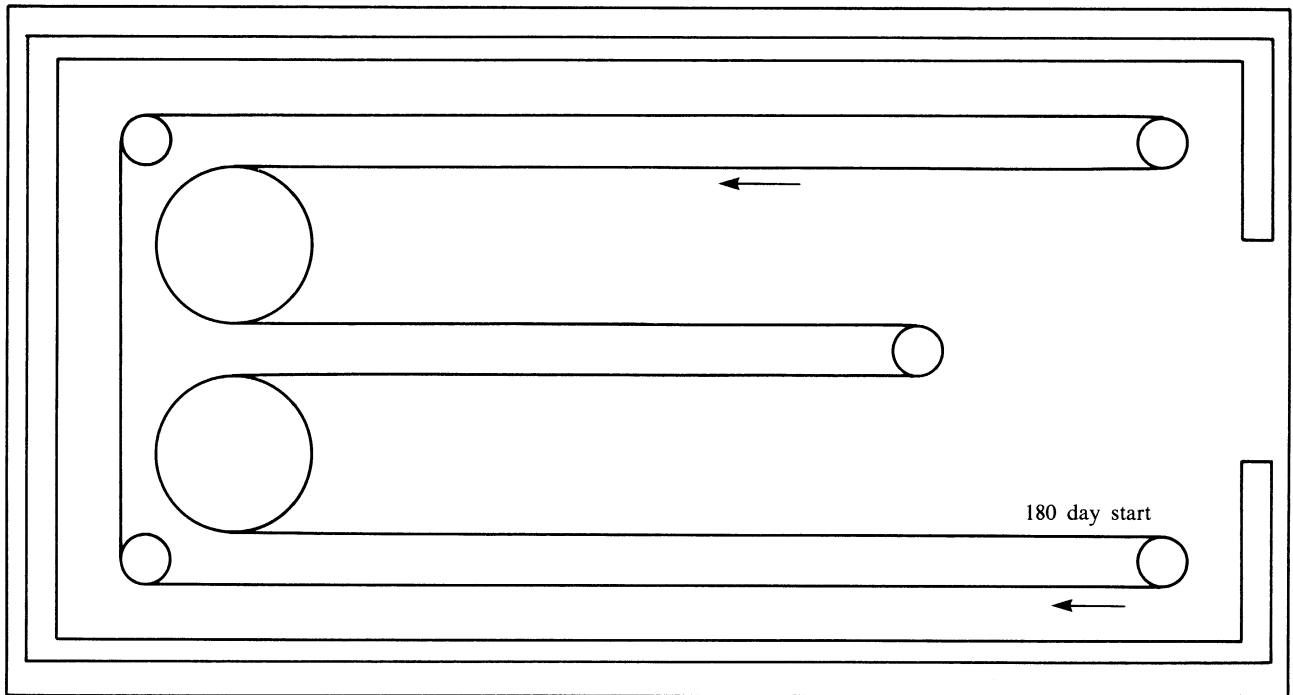
Figure 6.5-13.—*Schematic of conveyor system used to rotate eggs through the chamber during the 180-day chill period.*

colonized strains. Eggs from masses chilled under natural conditions generally hatch in about 2 or 3 days by late March or April. Equipment for automatic dispensing of eggs onto diet has already been developed for other insects and is readily adaptable for handling gypsy moth eggs. Also, if large-scale production of sterile males is required, equipment used for other species can be modified for harvesting.

The Expanded Mass-Rearing Facility

The previously described facility was adequate for rearing up to 10,000 gypsy moths per day. However, the production capacity of the facility was not adequate to meet both immediate program needs and anticipated needs for pilot testing the sterile male technique. Other disadvantages existed with the prototype facility. For example, because mobile

homes were used, the entire floor of the facility was elevated 0.6 m above that of the main building, making it necessary to use ramps to enter or leave the facility. The facility was difficult to sanitize because cabinetry, equipment, and shelving for holding insects were relatively immobile. Doorways were too small to permit carts and other equipment to move freely within the facility. Also, the floors and floor covering were inadequate for supporting heavy traffic for prolonged periods.

The prototype facility is currently being replaced by a more efficient facility that provides a fivefold increase in production capacity—50,000 insects per day. Moreover, if necessary, the capacity of the new facility can be easily expanded to permit rearing of 100,000 insects per day by simply building additional holding rooms.

Design and Construction

The expanded rearing facility (fig. 6.5-14) involves about 370 m² of floor space. The entire facility was designed to utilize the existing concrete flooring of the main building. All components except for an experimental rearing room (6A) and a walk-in refrigerator used for egg chilling are essentially wood frame construction. The walls and ceilings are gypsum board coated with a semigloss based paint. Deck and floor enamel was applied to all floors except the diet preparation room, which was resurfaced with an epoxy coating.

The entire facility, including the corridors, is equipped with HEPA filters (95 percent efficient) to minimize the incidence of airborne (viable and nonviable) contaminants.

Functional Components and Workflow

The components of the rearing facility (fig. 6.5-14) include a cool (8-10° C) room (1) for bulk storage of perishable diet ingredients. A reach-in commercial freezer (1.68 m³ is used for bulk vitamin storage and for holding preweighed batches of dry ingredients prior to diet preparation. An adjacent area (2) of the cool room is used to weigh out various diet ingredients. A room for temporary storage of rearing containers (3) is located adjacent to the diet preparation room (4), where containers are loaded onto trays (30 per tray) and prepared diet is dispensed into each container. Trays of containers with diet are placed on mobile carts and wheeled into an adjoining room (5) for infesting. Larvae are infested at 15 per cup or 450 per tray. Each cart is designed to hold 28 trays, or 12,600 larvae.

Carts of newly infested larvae are moved into the rearing rooms (6 or 6A) and sequentially circulated each day around the room. Insects are returned back to the immature-stages handling room (5) for transfer to new diet, sexing, or pupal harvest. Pupae used for obtaining adults are transferred to the adult handling room (9). After mating and oviposition, the moths are
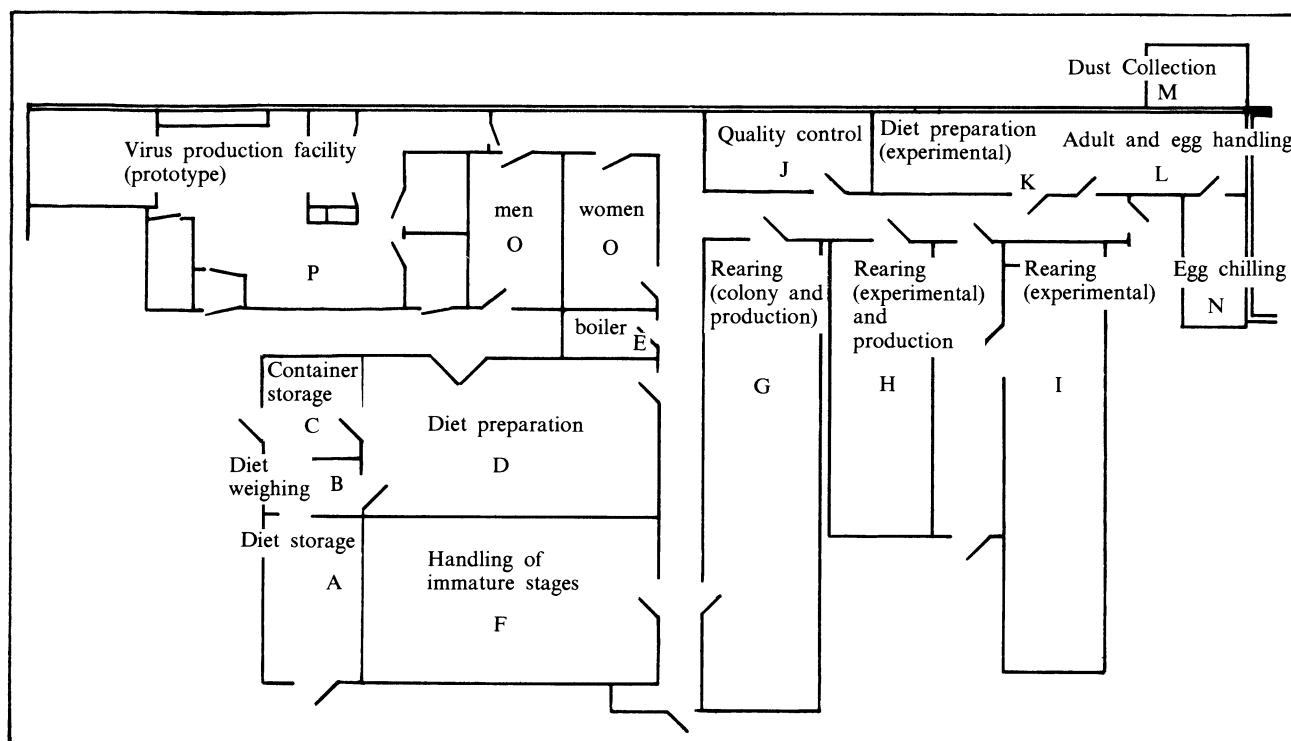
Figure 6.5-14.—Layout of the expanded gypsy moth rearing and virus production facility.

removed by the cyclone separator and dust collection equipment (9A) described earlier. After harvest, eggs are held until 28 days old in the adult- and egg-handling room before entering the chilling chamber (10). After chilling, the eggs are returned to the adult- and egg-handling room for surface disinfection.

Also, laboratories for conducting routine quality control measurements (7) and for preparation of experimental diets (8) were built into the new facility.

Environmental Control

The rooms for diet storage and weighing (1 and 2), rearing (6, 6A and 6B), adult and egg handling (9), and egg chilling have independent environmental control equipment. Rooms 3, 4, and 5 share a common air-conditioning and handling system. The environment is adjusted for worker comfort because insects are not reared in these areas. All locations where insects are reared (rooms 6, 6A, 6B, and 9) are equipped for rather precise control of temperature, relative humidity, light, and airflow. The airflow within the rearing rooms is designed for one change of air per 2 minutes. To provide uniformity of airflow in the rearing rooms, conditioned air is discharged from a main duct and forced through a false pegboard ceiling into the interior of the room. The air then circulates to the floor level and exits from the room via a plenum between the main (supporting) wall and a second inner wall that is elevated a few centimeters above the floor (fig. 6.5–15). Ducts situated along the ceiling pick up the air circulating between the walls and return it to the main duct. The airflow system is a modification of a design described earlier by Klassen and Gentz (1971), in which a series of return ducts were situated in parallel between the interior and exterior walls of the room. It is believed the system described here will provide equivalent uniformity of airflow at a lower cost.

Evaluation

As of January 1978 all components of the new facility, including the adjoining virus production facility (12), were fully operational. Room 6B of course, is the old prototype rearing room described earlier, which has been left essentially intact.

The operational part of the new facility has functioned satisfactorily so far, except for the dust-collecting equipment used to remove moths in the adult handling room. A new airflow system designed to reduce the ambient noise level within the room when the centrifugal blower is operating also reduced the vacuum capacity of the dust-collecting equipment. Therefore, some modifications of the unit will be necessary to obtain an optimal system for removing the moths.

Also, the original design did not include adequate provision for shipping of insects or a room for changing footwear and other clothing before entering the main rearing facility. Plans are being made to provide these needs.

The final cost of the new facility has been estimated at $60,000, about $161 per square meter. The entire complex of facilities including the new rearing facility, restrooms with showers, and the prototype virus production facility was constructed at a cost of $100,000. With minor modifications, these facilities are adequate to continue research and development towards an optimal mass-production system for the gypsy moth and the nucleopolyhedrosis virus. Also, the expanded facility should be able to meet the ever-increasing demands for insects to carry out the various laboratory and pilot-scale field tests required to develop an integrated pest management system for the gypsy moth.

Summary

Within a tight time frame of 3 years considerable progress has been made toward development of an efficient system for mass production of gypsy moths. During this period a concerted effort by SEA and APHIS personnel resulted in the procurement of a building (3,000 m³), the construction of laboratories, the development and evaluation of a prototype rearing facility, and its subsequent replacement with a new, expanded facility capable of rearing up to 50,000 gypsy moths per day. Special equipment for diet processing and for rearing and handling of the various stages of the insect was procured, evaluated, modified or constructed, and subsequently implemented to provide a more efficient production system.
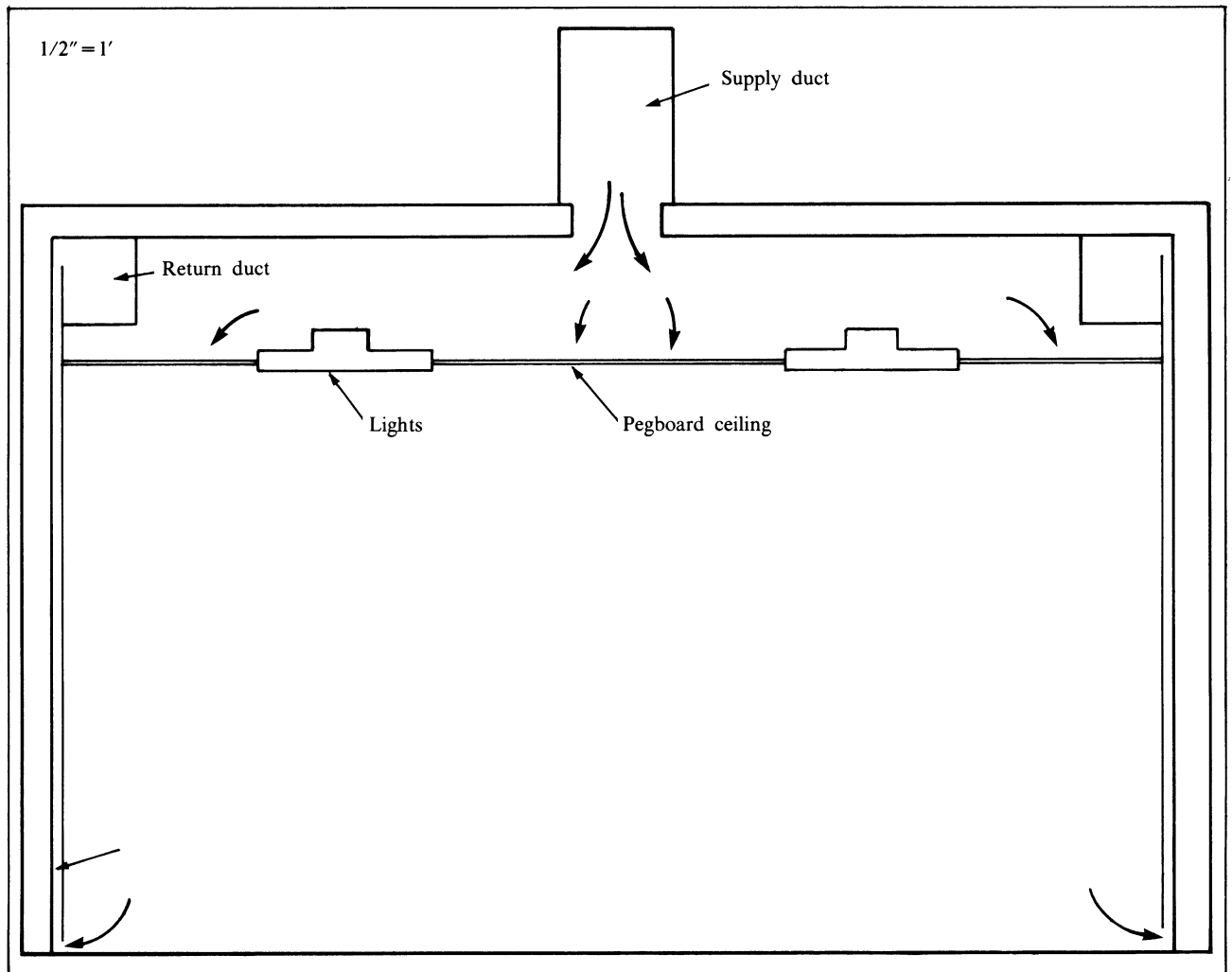
Figure 6.5-15.—*Cross section of rearing room showing the discharge of air through a pegboard ceiling and exhaust via a second inner wall.*

Methods were developed to insure that wild insects used for colonization, laboratory and field tests are vigorous and relatively disease free. The development and use of highly effective procedures for disinfection of egg masses along with rigid control of the rearing environment (humidity, ventilation, containerization, and clean air) have permitted the rearing of millions of insects annually without encountering significant problems with disease or incidental contamination.

The development of a simplified, low-cost diet, use of inexpensive containerization, and the reduction of labor requirements have reduced the cost of rearing gypsy moths for production purposes to less than $20 per thousand or about one-fourth of the cost prior to the inception of the expanded program. Since the cost reductions cited here were attained with manual labor, the implementation of available automation should permit the production of gypsy moths (for most purposes) for $10 per thousand or less.

Additional work is in progress to determine the optimal conditions for terminating the egg diapause and for promoting synchronous hatch. A New Jersey

strain is being selected for synchronous hatch after only 60 days of chilling. Such a strain would permit rearing of one generation per 4–5 months instead of the present turnover period of 7–8 months.

Further work needs to be focused on determining the optimal nutritional and environmental requirements for propagation of colonies. Heretofore, attention has been devoted primarily to diets and conditions for rearing insects for virus, parasite, and sterile male production.

More attention needs to be devoted to the development of practical quality control procedures to monitor continuously key components of the production system and the quality of the product. The development of a standardized rearing procedure in which human error can be minimized is necessary to insure the sustained production of insects of uniform quality. Therefore, critical operations such as the mixing and cooking of diet ingredients need to be automatically controlled and monitored to insure day-to-day uniformity of the diet. These matters will be given additional attention.

Although there is a need for additional research in the areas cited above, we believe that the rearing technology for the gypsy moth has been sufficiently improved to permit the field testing of biological and autocidal control methods at an acceptable cost.

In Vivo Mass Production of Gypsy Moth Nucleopolyhedrosis Virus

Martin Shapiro, Robert A. Bell, and Charles D. Owens

Introduction

At present, the cost of producing nucleopolyhedrosis virus (NPV) for field usage would approximate $60-$80 per 1,000 larval equivalents (LEQ) (Smith et al. 1976). Therefore, field applications per acre cost from $12-$40 when applied at the currently used doses of one to five times 10^{11} PIB's per 0.4 ha.

A major part of the high cost of producing NPV has been due to limited efficiency of techniques for rearing the host caterpillars. Limitations in the development of mass rearing technology have therefore restrained efforts toward development of efficient methods of virus production (Hedlund and Yendol 1974, Lewis 1971, Smith et al. 1976). As more efficient methods of host production evolved, it became feasible and desirable to develop more efficient procedures for in vivo production.

In Vivo vs. in Vitro Production

Tissue culture virus production is an exciting and challenging field and offers much potential. At the present time, however, production in the host caterpillar (in vivo) offers a more efficient means of production. Since it has been shown that it is feasible to produce 6×10^9 PIB's per insect for less than $0.03, it is difficult to envision such an efficient in vitro system. For certain purposes, however, the tissue culture system may offer some advantages and is discussed later (see section on Virus Inoculum).

Previous in Vivo Production Efforts

Prior large-scale production efforts have been reviewed elsewhere in this volume and will only be summarized briefly. With the exception of the Otis effort (discussed in chapter 6.3), previous NPV productions were performed via contracts. A brief review of these efforts is informative, as it illustrates an evolutionary process.

Initial contracts to Pennsylvania State University and Boyce Thompson Institute did not specify a required amount of virus to be produced, only the number of cadavers to be harvested. Subsequent contracts to Syracuse University and Bio Serv, Inc., specified the amounts of virus and not the number of larvae. This distinction becomes important, as the virus produced is the goal, not the number of diseased larvae. For example, late-instar larvae produce more virus than those inoculated in earlier instars; that is, as older larvae are utilized, the yield of virus per larva increases. Thus, Smith et al. (1976), utilizing larvae inoculated in the late third instar, obtained about 9×10^8 PIB's per larva, while 20×10^8 PIB's were obtained per larva inoculated during the fourth instar (See Production Methods).

Optimization of NPV Production

Our ultimate goal was to reduce NPV production costs to $10 per 1,000 larval equivalents (LEQ) by determining and optimizing the factors influencing virus production (fig. 6.5-16).

From a cost-efficiency standpoint, an optimal production system was one that produced the greatest amount of active virus per larva from a minimum virus dose in the shortest time possible. In addition, contaminant levels should be minimal.

In contrast to prior production schemes (Hedlund and Yendol 1974, Smith et al. 1976), NPV-induced larval mortality was not the sole criterion for time of harvest. One must take into account the rate of larval growth after inoculation with a given virus dose and its influence upon selection of a harvest time. It must be assured that all insects are infected in order to maximize production. Although different virus concentrations may lead to this response of the host population, it is important to consider the effect of the virus upon insect growth. When the dose is too high, above an LD_{100} level, larval growth is often inhibited, and insects die too quickly to permit the maximum virus yield per insect.

After the larvae are infected with NPV, the virus multiplies throughout the susceptible tissues of the host, resulting in death. Although the virus yield per insect increases during the infection period, it becomes more difficult to collect virus when all larvae have died; they frequently rupture and release virus into the diet and feces, making harvest difficult. It is important to minimize the time from virus inoculation to harvest, because the shorter the period, the greater the amounts of insects and NPV produced per unit of time, which lead to a reduction in costs.
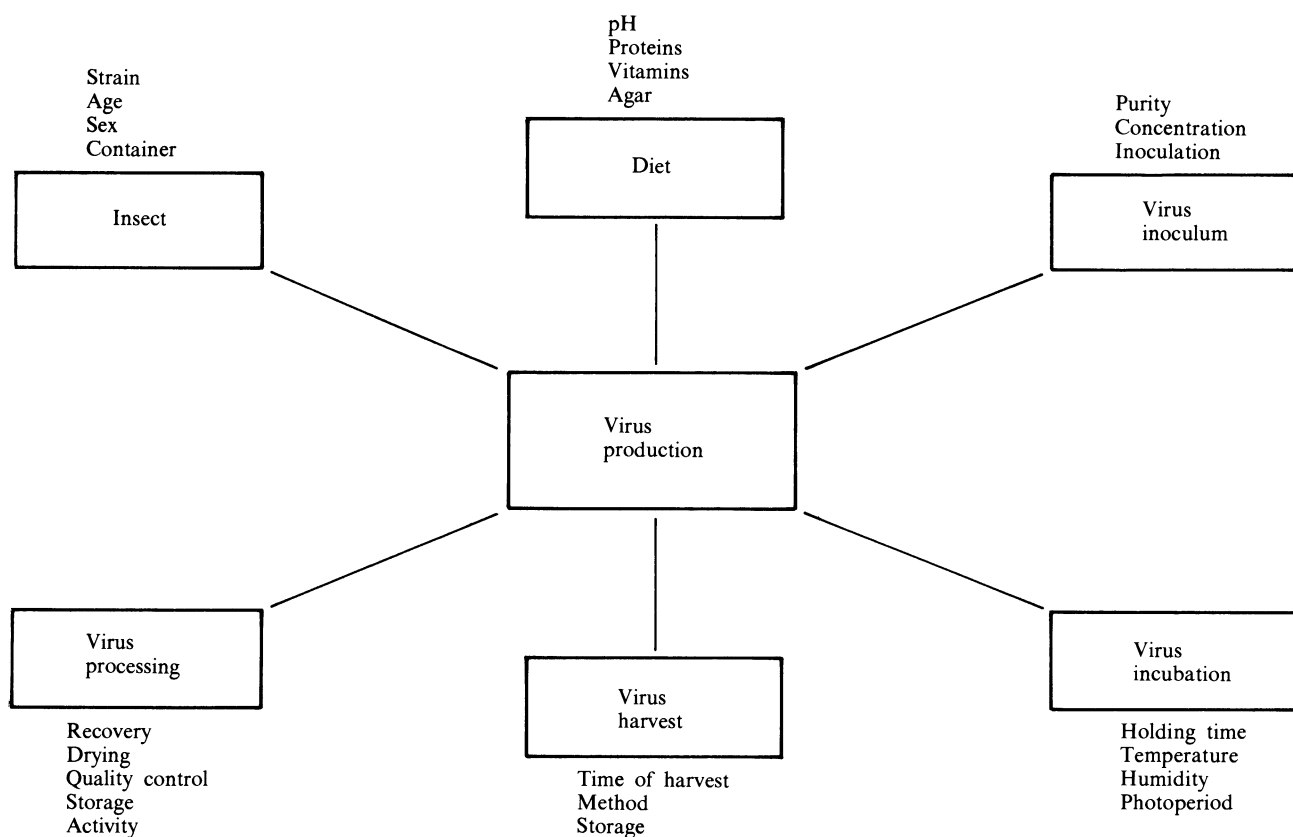
Figure 6.5-16.—*Factors involved in virus production.*

Before initiating research to define optimal conditions for NPV production, standards had to be established regarding the insect, diet, and virus inoculum.

The Host Insect

Wild vs. Colonized Insects

Field-collected insects have been utilized for bioassays (Doane 1967, Magnoler 1970b, 1974) as well as for virus production (Hedlund and Yendol 1974, Smith et al. 1976). These workers used feral insects, not only because of their relative availability and relatively low cost of collection, but also because sufficient numbers of colonized insects of suitable quality were not available. There are several disadvantages in using wild, field-collected insects. Wild populations frequently have a high incidence of parasitism and NPV. To minimize these problems, Magnoler (1970b) obtained egg masses from lightly infested areas. Hatchability of field-collected insects is usually reduced after several month's refrigeration under laboratory conditions, making year-round availability an impossibility. It may be difficult to establish a "wild" population in the laboratory on artificial diet, and growth and development of the larvae may not be uniform enough for efficient virus production. Within the past year, the New Jersey colonized strain (now in the 17th generation) has been utilized by other workers for both production and research because of its availability on a year-round basis, vigorous and predictable growth, predictable response to NPV, and apparent freedom from disease.

Although the establishment and perpetuation of a laboratory culture do not necessarily guarantee a virus-free culture, continuous culture should certainly reduce the amount of available virus (dilution effect). Until a sensitive tool for diagnosis of virus at low levels is established, the prime criterion for the presence of virus, unfortunately, remains virus-induced mortality. Sanitation procedures and egg disinfection should be practiced vigorously, in order to reduce or eliminate NPV and other contaminants. Doane (1969, 1975) demonstrated transovum transmission of gypsy moth

NPV and the effectiveness of sodium hypochlorite as a viricidal agent. Magnoler (1970, a, b) utilized formalin as a surface disinfectant for egg masses. When the two chemicals were compared it was concluded that formalin (1:10 dilution, 60 minutes) was more effective than hypochlorite (1:10 dilution, 60 minutes). Since formalin treatment of egg masses was initiated at Otis Air Force Base, overt virus incidence has been virtually eliminated.

The Diet

Considerable attention has been devoted to the development of a simple and economical diet for rearing host larvae for NPV production. In a series of preliminary tests, the modified hornworm diet (Yamamoto 1969) was shown to be excellent for NPV production and has been utilized as the standard.

The Virus Inoculum

The virus inoculum used in pilot production run was the Hamden isolate, which has been used widely for both bioassay and production (Hedlund and Yendol 1974, Lewis 1971, Magnoler 1970b, Smith et al. 1976).

Virus Activity

Magnoler (1970b) compared the *L. dispar* NPV Hamden isolate with those from France, Yugoslavia, and Italy and concluded that the Hamden isolate had the greatest activity. Using the same isolate, Magnoler (1974) developed a reproducible bioassay. LD_{50} values obtained by Doane (1967) and Magnoler (1974) were quite similar—1.04×10^3 and 1.32×10^3 for third-instar larvae. These results might seem surprising, but Connecticut isolates were used by both workers. Activities of viruses obtained from different geographical areas may or may not be similar. Recently, the activities of the Hamden isolate and one obtained from Trenton, N.J., were tested, and both were found to be similar.

Activity, as important as it might appear to be, is not the sole criterion for the selection of a production isolate. Virus yield is of paramount importance and may or may not be equated with activity.

Unfortunately, no data appear to be available comparing different *L. dispar* NPV isolates for both activity and yield.

Purity of Virus Inoculum

Virus inoculum may be obtained from either in vitro tissue culture or in vivo host production. At present, tissue culture produced virus, either nonoccluded free virions and/or PIB's, represents bacteria-free, noncontaminated inoculum. In this regard, it would be quite feasible to utilize in vitro produced material as primary inoculum. In vivo produced inoculum is not bacteria free and may contain as many as 10^9 aerobes per gram or more (see Quality Control). The number of bacterial contaminants per gram of inoculum may or may not create a safety problem, as long as pathogens such as *Salmonella* and *Shigella* are not present. In some instances, hemolymph from virus-infected insects results in "clean" inoculum.

Virus Storage

A sufficient quantity of virus inoculum should be available for an entire production run. Ideally, the inoculum should be available as a powder (air dried or freeze dried) and divided into production aliquots. Each aliquot or sample should contain enough virus for a single day's production. Two criteria should be met during storage: Viral activity should be maintained, and microbial contamination levels should not increase. Storage of a virus suspension, even under refrigeration, might not result in loss of activity but could lead to an increase in contaminant levels. Frozen suspensions of virus might be acceptable under both criteria, and infective hemolymph and tissue culture produced viruses are often stored in this manner. Powders are obtained primarily by air drying or freeze drying PIB suspensions, and spray drying should not be overlooked. Although acetone extraction or acetone-lactose coprecipitation has been employed (Dulmage et al. 1970), evidence exists that these treatments result in a loss of activity, even after storage as powders (Ignoffo and Shapiro 1978).

Production Methods

Large-scale in vivo virus production is dependent upon efficient mass rearing of the host insect. Within the past few years, much progress has been made in rearing technology. Lewis (1971), using laboratory-reared insects, envisioned an annual production of 1 million larvae with a theoretical yield of 2×10^{15} PIB's per year. During spring 1977, 1×10^{15} PIB's were produced from 500,000 larvae in 7 weeks by ARS—APHIS personnel (see In Vivo Production at Otis Air Force Base in chapter 6.3). Although this effort was a crash program involving daily production, it would have been feasible to produce 7.2×10^{15} PIB's 5 days per week using existing technology. As more efficient production technology through mechanization and greater virus yields per insect is obtained, greater production efficiency will certainly be achieved. The remainder of this chapter will evaluate production methodology as well as summarize research accomplishments.

Inoculation of Larvae

A suspension of NPV can either be incorporated into the artificial semisynthetic diet or simply placed on the diet surface; both methods of inoculation have been used in production. Ignoffo (1966), in an excellent review on virus production, described his work with *Heliothis zea, H. virescens,* and *Trichoplusia ni* in which virus suspensions were sprayed on the diet surface. Hedlund and Yendol (1974) and Lewis (1971) incorporated *L. dispar* NPV into the diet during diet cooling, at 47° C and 55° C, respectively.

In the case of the gypsy moth, surface treatment has some advantages over diet incorporation, in that less inoculum is required to attain infection. Diet can be prepared in the regular diet processing area and transferred to virus production for inoculation, thus avoiding duplication of diet preparation equipment. Also, by introducing virus on the surface of the diet after cooling, as opposed to incorporation into warm diet, there is less chance of viral inactivation by heat. Diet temperature can play a critical role in virus inactivation, as Rollinson (1977) found that virus

inactivation occurred at diet temperatures greater than 58° C. Thus, diet should be cooled below 58° C before virus is added.

The effectiveness of these two methods is compared in table 6.5-16. The mortality obtained at 10 days post inoculation and the virus yield per larva were much greater when the inoculum was applied to the diet surface.

Time on Infected Diet

From an economic standpoint, infected larvae should remain on virus-contaminated diet through-out the production period. If larvae are transferred to fresh, uncontaminated diet, additional costs are incurred because of extra diet, containers, and labor. During production of cotton bollworm and cabbage looper NPV's, larvae remained on virus-treated diets (Ignoffo 1966).

For the gypsy moth, Lewis (1971) removed old diet and added fresh, uncontaminated diet as needed. Larvae were fed virus-treated diet for 48 hours, at which time fresh, virus-free diet was offered (Smith et al. 1976). Throughout the production run, infected larvae remained on virus-contaminated diet. A significant relationship was observed between the time of larval feeding on virus-treated diet and the virus concentration in the diet (Hedlund and Yendol 1974). The data are interesting but inconsistent. Virus yield was similar when larvae were exposed to virus-treated diet for 48 hours or continuously, but when larvae were exposed to virus-treated diets for 96 hours, virus yield was greater.

In the studies, no differences were found in either virus yield or virus kill, when larvae fed on virus diets for more than 24 hours (day 10 harvest). When the test

period was extended to 15 days, different feeding periods did not result in different virus yields or virus-induced mortality (table 6.5-17). From these data, it would appear that enough virus is consumed within the first 24 to 48 hours to result in 100 percent infection.

Optimal Time of Inoculation

The objective of NPV production is to utilize host tissue as efficiently as possible and to obtain the maximal yield of biologically active virus per insect. In the case of the tobacco budworm, 6-day-old larvae were used to propagate NPV (Ignoffo 1966). It was believed that insects older or younger would not produce maximal yields of virus, but only mortality data are presented. Cabbage looper larvae 6 to 7 days old were used, when 70 percent of their larval development had been completed. The use of later instar larvae accomplished several objectives. The ingestion of a lethal dose occurred within a short period, maximum utilization of larval tissues were obtained, and various phases of virus production (larval rearing, infecting, and harvest) were easily systematized (Ignoffo 1966). Lewis (1971) inoculated second- and third-instar gypsy moth larvae for NPV production and obtained 2×10^9 PIB's per larva.

Table 6.5-16.—*Mortality and NPV yield when inoculum was applied to the surface vs. incorporation in the diet.*

Treatment	Percent mortality[1]	PIB's per larva
Surface	28.0	1.49×10^9
Incorporation	2.8	4.86×10^8

[1]Virus-induced mortality at 10 days postinoculation, 29° C; fourth-instar larvae.

Table 6.5-17.— *Effect of feeding period on virus yield and virus mortality[1]*

	Day of harvest			
	Day 10[3]		Day 15[3]	
Days on virus diet[2]	PIB's per larva ($\times 10^9$)	Percent kill	PIB's per larva ($\times 10^9$)	Percent kill
1	1.32	17	1.89	96
2	1.70	21	1.98	98
3	1.97	24	1.95	95
4	1.89	31	1.89	97
5	1.96	22	1.71	97
6	2.01	21	1.93	95
7	1.60	21	1.94	96
10	1.59	27	—	

[1]Larvae infected in fourth instar at 29° C.
[2]Following exposure to virus diet, insects were transferred to fresh, uncontaminated diet.
[3]Each day harvest is represented by a different set of larvae.

Smith et al. (1976) inoculated late third-instar larvae and obtained about 9×10^8 PIB's per larva. Hedlund and Yendol (1974) studied the relationship of virus yield to inoculation time, dosage, and larval weight; the greatest virus yield per insect was obtained when larvae weighing 400–499 mg were fed on a diet containing 1.5×10^5 PIB's per milliliter of diet. The average yield per insect was 1.64×10^9 PIB's, with a maximum of 2.23×10^9. These authors concluded that maximal production was 1.56×10^6 PIB's per milligram of final larval weight. It is difficult to equate Lewis' (1971) results with those of Hedlund and Yendol (1974) or Smith et al. (1976) regarding virus yield. If maximal yield of NPV is about 1.5×10^6 PIB's per milligram of final weight of insects, how can the yield from larvae inoculated in second to third instars (Lewis) be comparable to that of the insect inoculated in the fifth instar (Hedlund and Yendol) during the same time period (about 15 days)?

Data (fig. 6.5–17) indicate that larvae inoculated in the later instars produced more virus than those infected in the earlier instars. To obtain 1×10^9 PIB's per larvae, it was necessary to infect larvae in the fourth or later instars. When fifth- or sixth-instar larvae were infected, it was possible to obtain greater NPV yields than with fourth-instar larvae. The greatest yields were obtained with females, which in general complete six larval instars, compared to five of the male.

During the large-scale production run, 500,000 fourth-instar larvae were utilized, for a total of 1×10^{15} PIB's ($\bar{x} = 2.04 \times 10^9$ per larva). The results were pleasantly surprising in that the production efficiency

$$\frac{\text{PIB's per larvae}}{\text{weight of larva (mg)}} = 2.29 \times 10^6$$

was much higher than the 1.56 obtained by Hedlund and Yendol (1974), although the Otis larvae were smaller than the Pennsylvania larvae. It is difficult to analyze these differences because the diet, the source of insects, and the method of inoculation were different. In all likelihood, a combination of diet and host insect played a more critical role than the method of inoculation.
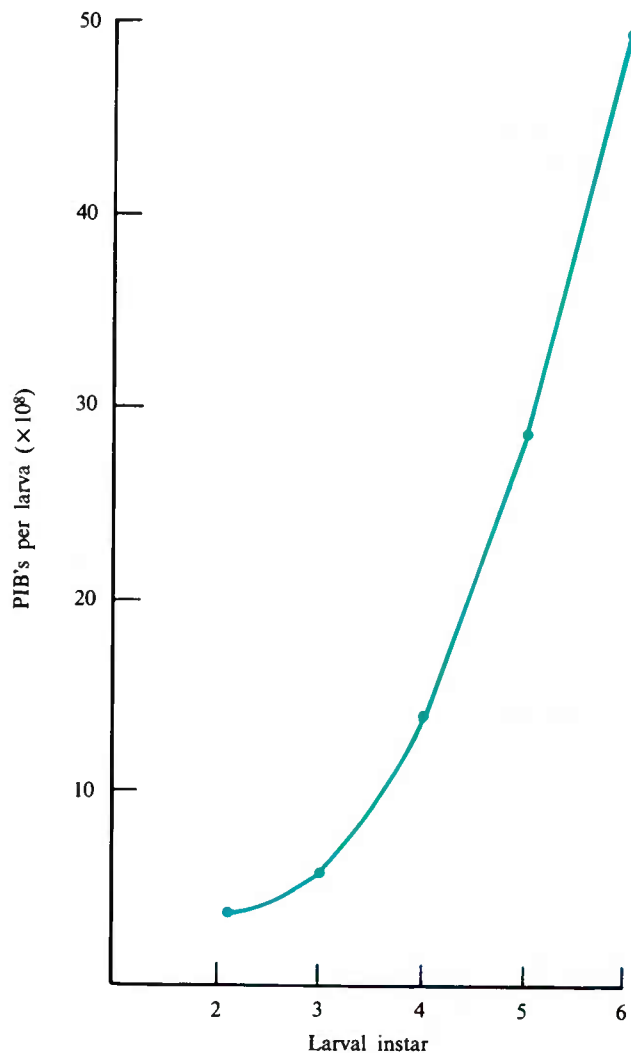
Figure 6.5–17.—*Age of insect vs. virus yield.*

Males vs. Females

In all previous production efforts, larvae were chosen irrespective of sex (Hedlund and Yendol 1974, Lewis 1971, Smith et al. 1976). During the pilot plant production run, a mixed population of fourth-instar male and female larvae was used.

Each day for 38 days, 10 living infected males and 10 living infected females were removed, and virus yields were determined (table 6.5–18). It is quite apparent that a greater yield of virus was obtained from female larvae. The female is, in general, no more

Table 6.5–18.—*Virus production from male and female larvae[1]*

Larval stage when inoculated	Mean PIB's per larva ($\times 10^9$)		PIB's per milligram ($\times 10^6$)	
	Male	Female	Male	Female
Fourth	1.57	3.99	1.85	1.78
Fifth	1.40	4.96	1.73	2.10

[1]Day 10 harvest, 29° C.

Table 6.5–19.—*Virus yield and larval weights as an expression of female:male ratios[1]*

Female:male ratio	Mean weight (ml)	Mean NPV yield (1×10^9)	PIB's per milligram ($\times 10^9$)
75:25	1,614	2.610	1.62
54:46	1,460	1.956	1.34

[1]Larvae infected in fifth instar.

efficient than the male, because no more virus is produced per milligram of larval weight, but because the female attains a greater biomass than the male, more virus per larva is produced. These data indicated the potential advantages of female larvae as the preferred host for NPV production.

When fifth-stage male and female larvae were separated, at day 21, and inoculated with NPV, two facts emerged: Greater yields of virus are produced in females, and females are more susceptible to virus than males.

Virus Yield

It was shown previously that females infected in the fourth instar produced more virus than males (table 6.5–18). The same trends occured in larvae infected as fifth instar (table 6.5–18). Total virus produced per insect was always higher in females, and it was not uncommon to obtain 6×10^9 PIB's per female larva. Moreover, the larger the female, the greater was the yield of virus produced per insect.

When mixed populations of fifth-instar male and female larvae were used for virus production, the total yield was largely dependent on the ratio of females to males. As the ratio of females to males increased, the yields and total larval weights increased (table 6.5–19).

Sensitivity to Virus

Campbell (1963*a, b*) observed that female larvae were more sensitive to NPV during late instars, in that more males were found following an epizootic than females (Reiff 1911). In a series of experiments, fifth-instar male and female larvae were challenged with an LD_{100} and noted that the mortality rate was quite

different between the sexes (fig. 6.5–18). By day 13, only 10 percent of the males had died from NPV, while 34 percent of the females were virus killed. In general, females died 5 days earlier than males.

Are males inherently more resistant, or, conversely, are females more susceptible? Female larvae undergo one more molt and pupate later than males and, therefore, have a longer larval feeding period. When males and females are infected during the fifth instar, males can still pupate, if the period from virus inoculation to pupation is less than the lethal infection period (for the virus). When fifth-stage females were infected with a high virus concentration, larval death invariably occurred. In this instance, the lethal
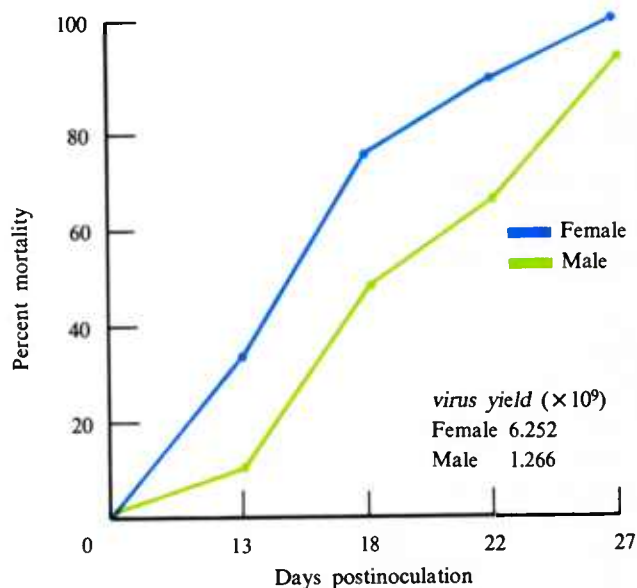
Figure 6.5–18.—*Virus mortality and virus yield from male and female larvae.*

infection period was shorter than the time required for pupation. When females were inoculated during the last instar, pupation could still occur.

Optimal Inoculum Dose

For large-scale production, a uniform response to the virus is required—100 percent infection or possibly 100 percent mortality. Although each virus production system is unique, doses of virus inoculum have varied from 1×10^5 to about 4×10^6 in all described systems (cotton bollworm, 3.6×10^6; cabbage looper, 1×10^6 (Ignoffo 1966); and Douglas-fir tussock moth, 1×10^6 (Chauthani and Claussen 1968). For gypsy moth NPV production, concentrations of 1×10^5 (Hedlund and Yendol 1974, Lewis 1971), and 3×10^6 (Smith et al. 1976) were used.

The relationship between inoculum dose and virus yield must be determined for each production system. In the pilot production, an inoculum dose of 5×10^6 PIB's per milliliter per container was used. This dose permitted the desired larval growth and virus yield. Concentrations greater than 5×10^6 did not necessarily result in greater virus yields, and doses less than 1×10^6 produced less virus. In production involving fifth-instar females, 5×10^6 has also been utilized successfully as the inoculum dose.

Environmental Effects

Temperature

Temperature appears to play a critical role in the course of epizootics of gypsy moth NPV. Vasiljevic (1958) concluded that high daily temperatures favored the development of virosis. Orlovskaja (1964, 1967), an advocate of latent infection, stated that virus outbreaks were readily activated at high rearing temperatures. In general, studies of *L. dispar* NPV have been carried out at 23°–25° C (Doane 1969, Lewis 1971, Magnoler 1970*b* and 1974, Nappi and Hammill 1975). Yadava (1970) investigated the relationship of temperature and humidity upon the course of virus disease. When the temperature increased up to 26.5°–27.5° C, the lethal infection period decreased. As the temperature increased to 29°–31° C, virus development was not enhanced.

For routine rearing in the laboratory, larvae are maintained at 25°–26° C. To determine the optimal temperature for NPV production, larvae were held at 23°, 26°, 29°, or 32° C after virus inoculation. The effect of temperature was evaluated with respect to mortality, virus yield, lethal infection periods and the biological activity of virus extracted from the test insects. NPV yields and viral activity were similar from insects reared at 23°, 26°, or 29° C, when virus was harvested from virus-killed larvae (table 6.5–20). Thus, 29° C seemed to be optimal, as highest yields could be obtained in the shortest time (table 6.5–21).

As the temperature increased, the LT_{50} (the time required to achieve 50 percent mortality of the test population) decreased. When virus was harvested at day 10, the maximal yield was obtained at 29° C. Again, a higher temperature (32° C) resulted in lower virus yield. The results were in general agreement with Yavada (1970); as temperature increased, the lethal infection period decreased. In program studies, however, virus-induced mortality was faster at 29° or

Table 6.5–20.—*Effect of postinoculation holding temperature on NPV yield and activity*[1]

Temperature (° C)	PIB's per larva	Activity (PIB's to obtain LD_{50}[2])
23	1.14×10^9	5.0×10^3
26	0.97×10^9	7.0×10^3
29	1.13×10^9	4.0×10^3
32	0.35×10^9	8.5×10^4

[1]Larvae inoculated in late third instar; yields based on virus-killed larvae.

[2]Bioassay against second-instar larvae.

Table 6.5–21.—*Effect of temperature on rate of NPV-induced mortality and incidence of mortality and yield at 10 days postinoculation*[1]

Temperature (° C)	Time of LD50 (days)	Percent mortality	PIB's per larva
23	22	8	0.70×10^9
26	19	18	1.30×10^9
29	10	44	2.76×10^9
32	10	54	0.48×10^9

[1]Larvae infected in fifth instar.

32° C than at 26° C. Virus-induced mortality, the sole criterion utilized by Yavada (1970), is not necessarily more significant than virus yield. Although virus-induced mortality was more rapid and greater (day 10) at 32° C than 29° C, yield *and* activity were much lower at 32° C. The higher temperature apparently had a detrimental effect upon virus replication.

Humidity

Wallis (1957) concluded that an increase in humidity increased virus incidence. In the laboratory, high relative humidity conditions resulted in high NPV incidence; low humidity led to low NPV incidence. Orlovskaja (1964, 1967) reported that high temperature, excessive humidity, starvation, or feeding with unusual food activated occult virus.

On the other hand, Yadava (1970) demonstrated that relative humidity had little effect upon virus replication, while temperature played a critical role. Program studies showed little difference in either the *rate* of virus-induced mortality or *total mortality* at low (34–36 percent) or high (60–62 percent) relative humidities at 29° C. For virus production, relative humidities of 50–60 percent are used, at a temperature of 29° C. With lower humidities, the rearing diet dries excessively and would have to be replaced.

Photoperiod

Photoperiod has not been studied as a factor affecting the course of NPV. The gypsy moth is commonly reared in a 16-hour light: 8-hour dark cycle (Leonard and Doane 1966, Magnoler 1970*a, b,* and 1974, ODell and Rollinson 1966, Ridet 1972). Although photoperiod is not mentioned by Lewis (1971), Hedlund and Yendol (1974), and Smith et al. (1976), the light phase was presumably 16 to 17 hours. At the mass-rearing facility, the light/dark cycle is maintained at 16:8.

The effects of photoperiod by rearing insects in light:dark regimens of 16:8, 12:12, and 8:16 were compared. Virus mortality and yield were evaluated from larvae infected as neonates and reared under different light regimens, and insects reared under standard rearing conditions for 14 days and then

maintained under different photoperiods. No differences in larval growth, virus-induced mortality, or virus yield were observed.

Diet

The development of semisynthetic diets, pioneered by Vanderzant et al. (1962*a, b*), facilitated continuous mass rearing and virus production. The commercial production of the cotton bollworm NPV (Ignoffo 1966) and the Douglas-fir tussock moth NPV (Chauthani and Claussen 1968) became feasible because of improved rearing technology utilizing artificial diets.

Several diets have been developed for gypsy moth rearing (Leonard and Doane 1966, Magnoler 1970*a*, ODell and Rollinson 1966, Ridet 1972, Tardif 1975) with varying success. Some of these diets have also been used for virus studies and production—the *Leonard and Doane diet* (Doane 1969), *Odell and Rollinson diet* (Hedlund and Yendol 1974, Lewis 1971, Smith et al. 1976), and *Magnoler diet* (Magnoler 1970*b*, 1974). For the pilot production, the modified hornworm diet (Yamamoto 1969) was chosen.

One of the major objectives of the research program was to develop a simple, low-cost diet for NPV production. Recently, a simplified high wheat-germ diet was developed and has been utilized successfully for colony production. When the hornworm and simplified high wheat-germ diets were compared for virus production, little differences were noted. The high wheat-germ diet has some advantages in that it contains fewer dietary ingredients and is less costly.

At present, several gypsy moth diets are being evaluated—Leonard and Doane (1966), Magnoler (1970*a*), ODell and Rollinson (1966), and Ridet (1972), as well as a lima bean diet (Shorey and Hale 1965)—and compared with the hornworm and high wheat-germ diets. The modified hornworm diet was superior in all tests; and the high wheat-germ diet was superior to the others.

Reduction in dietary costs can be obtained in several ways: Use of less costly dietary ingredients,

reduction of essential and/or elimination of non-essential ingredients, and agar substitutes. Rearing insects for virus production required a less complex diet than for sterile male or colony production, because the single objective was good larval growth, not raising pupae, adults, or viable eggs.

Wheat Germ

Wheat germ is a major ingredient and a source of proteins, carbohydrates, minerals, lipids, etc. During the past year, wheat germ has been used from different commercial sources, depending primarily upon availability and cost. When Mennel®, Niblack®, and Kretschmer (Toasted)® were compared, Mennel® and Niblack® products were comparable and were slightly better than toasted Kretschmer®. Moreover, similar trends were observed among control insects. Wheat bran and wheat meal were inferior to wheat germ. Kretschmer Toasted® wheat germ has some advantages over the others: Toasted and vaccum-packed wheat germ has lower contaminant levels than the raw wheat germ and greater storage stability, and can be stored at room temperature with no loss in biological activity.

Casein

Casein has been used as the primary protein source in many insect diets (Vanderzant 1966) and in all diets for the gypsy moth. In a series of tests, different proteins and their hydrolysates were compared to casein (at equivalent protein concentrations) in the high wheat-germ diet (table 6.5-22). It is apparent that several proteins, namely trypticase (a casein hydrolysate), soy, phytone (a soy hydrolysate) and lactalbumin, are comparable to casein and could be utilized as primary protein sources. Torula yeast, a byproduct of the paper pulp industry, is almost as effective as casein and is an inexpensive source of protein. Cost and availability are important factors in the selection of protein sources for virus production.

Substitution of Wheat Germ

Although casein is the primary protein source in wheat germ/casein diets, wheat germ itself also provides protein. Therefore, the question arose as to whether casein could be replaced totally by merely increasing the wheat germ fraction (table 6.5-23). At the wheat germ concentration of 180 g per liter of diet, virus yield was comparable to the wheat germ (120 g per liter)/casein (36 g per liter) standard.

Although an 18 percent wheat germ diet alone might offer a possible alternative to wheat germ/casein, especially from a cost-efficiency standpoint, two problems exist regarding mass rearing and virus production: Contamination levels and potential for hydrolytic and/or oxidative changes leading to rancidity.

Vitamins

In general, the vitamins required for insect growth and development are the B-vitamins and ascorbic

Table 6.5-22.—*Relative effectiveness of various protein sources for NPV[1]*

Protein source	Efficiency of protein compared to casein
Casein	1.00
Trypticase	1.04
Soy	.98
Phytone	.99
Gelatin	.84
Gelysate	.72
Torula yeast	.83
Yeast Hydrolysate	.91
Lactalbumin	.97

[1]Larvae infected in fifth instar.

Table 6.5-23.—*Utilization of wheat germ as the sole protein source[1]*

Protein source	Relative efficiency of wheat germ compared to standard
Wheat germ and casein[2]	1.00
No casein, wheat germ only[3]	
50% of standard	.15
100%	.68
150%	.85
200%	.87

[1]Female larvae infected in fifth-instar.
[2]Standard diet: 120 gram wheat germ + 25 gram casein per liter.
[3]50 percent + 60 gram wheat germ per liter.

acid. These materials can be added to the diet as individual vitamins or as a prepackaged mix. In the classic bean diet (Shorey and Hale 1965), yeast is utilized as a B-vitamin source, and both brewers and torula yeast have been utilized interchangeably.

It would appear that the concentrations of vitamins used in the mass-rearing diets are also utilized in virus production diets (Chauthani and Claussen 1968, Hedlund and Yendol 1974, Ignoffo 1966, Lewis 1971, Smith et al. 1976). In program investigations, the high wheat-germ diet was employed, and the vitamin concentration was increased up to tenfold (table 6.5-24). A small increase in vitamin mix concentration resulted in little increase in virus yield, but a tenfold increase did result in 5.6×10^9 PIB's per larva, up from 4.4×10^9. Whether the increase is due to vitamins or other materials present in the vitamin mix is not known. The additional cost incurred, however, would not justify an increase in vitamins. At present, the substitution of torula yeast is being evaluated and preliminary results are promising.

pH of Diet

No mention of diet pH was made for the production of the cotton bollworm (Ignoffo, 1966), Douglas-fir tussock moth (Chauthani and Claussen, 1968), and gypsy moth NPV's (Hedlund and Yendol 1974, Lewis 1971, Smith et al. 1976). It must be assumed that diet pH plays little role in virus production. The omission of pH data is very striking, as pH for tissue culture is of paramount importance. The pH of artificial diets for lepidopterous insects, like that of tissue culture media, is acidic. The modified hornworm diet has a pH about 5.7 and has been utilized as both the rearing diet and the virus production diet.

In a series of experiments, the relationship between dietary pH and virus yield were compared (table 6.5-25). The hydrogen ion concentration (pH) was increased by $4 M$ KOH or decreased by $1 M$ HCl. In general, little difference in virus yield occured at pHs 4 through 7. As the pH increased to 8, virus yield decreased; and as the pH increased above 6, the level of microbial contamination increased.

Agar Substitutes

In general, agar has been utilized as the gelling agent in insect diets. Agar has been the most expensive ingredient in insect diets and has accounted for up to 50 percent of total dietary costs. As the price of agar has increased, attention has focused on agar substitutes. Calcium alginate (Moore and Navon 1969, 1973, Navon and Moore 1971); sodium alginate (Spencer et al. 1976), and carrageenan (Baumhover et al. 1977, Patana 1969, Vail et al. 1973) were utilized successfully for the rearing of several lepidopterous insects. Vail et al. (1973) suggested that carrageenan could be used as the gelling agent for production of the cabbage looper NPV.

All previous production of the gypsy moth NPV utilized agar as the gelling agent (Lewis 1971, Smith et al. 1976). As part of the research efforts the feasibility of carrageenans as agar substitutes was investigated. The most commonly used carrageenan, Gelcarin HWG, was comparable to agar at concentrations

Table 6.5–24.—*Effect of vitamin concentration on virus yield[1]*

Diet	Vitamin concentration[2]	NPV yield compared to standard
Hornworm	1×	1.03
High wheat germ (Std)	1×	1.00
	2×	.92
	4×	1.10
	10×	1.29

[1]Larvae infected in fifth instar.
[2]Vitamin premix (Hoffman-LaRoche Mix) contains inositol, dextrose, tocopherol, B-vitamins, vitamin A, vitamin C.

Table 6.5–25.—*Relationship between dietary pH and virus yield[1]*

Dietary pH	Comparison of NPV yield to pH 5.7 standard
4	0.89
5	.91
5.7	1.00
7	.87
8	.64

[1]Modified hornworm diet: larvae challenged as fourth instar.

ranging from 0.75 to 2.0 percent (weight/weight), indicating a potential usefulness of Gelcarin. Three other carrageenans were compared to agar at concentrations of 1 and 2 percent (w/w) and are being tested further because of their effectiveness and low price, as compared to agar (table 6.5-26).

Host Density

In the determination of the optimal density per container, one must consider the biology or behavior of the insect. In the case of the cotton bollworm, larvae were held in individual containers (Ignoffo 1966). Four Douglas-fir tussock moth larvae were maintained in a 30-ml clear plastic container for virus production (Chauthani and Claussen 1968). The gypsy moth can be reared individually for bioassay studies (Doane 1967, Magnoler 1970b, 1974). For virus production, however, insects were maintained in groups of 10 in 100×15-mm petri dishes (Hedlund and Yendol 1974) or 12 in 480-ml waxed cups (Smith et al. 1976).

For the pilot production, 10 fourth-instar larvae were maintained in a 180-ml container. This number was chosen initially because growth and development were normal, and crowding was not considered excessive. When larval density was related to virus yield per larva, the results were inversely density dependent (table 6.5-27). Although maximal yield per larva was accomplished with singly reared insects, the aggregate rearing approach is less expensive for virus production.

Subsequent research indicated that greater virus yields per larva per container could be realized with fifth-instar female larvae. These larvae can attain a size of 2-3 g at harvest, under optimal conditions of host density. For a given container system (180 or 240 ml), maximal larval size and virus yield were obtained from five females per container (table 6.5-28). As the larval density increased, both the larval weight and subsequent virus yield per larva decreased markedly. Moreover, the total virus yield per container was no greater at densities of 10 or 15 than at 5. At the present time, virus production is centered around five female larvae per 180-ml container. Under this production system, a minimum of 1×10^{11} PIB's (a 0.4-ha equivalent dose per application) can be obtained from only 20 larvae, resulting in an efficient production at the cost of about 2 cents per larva.

Harvesting of Infected Larvae

Time of Harvest

Ideally, the time of harvest should be that period when virus yield per larva and biological activity of

Table 6.5-26.—Comparison of agar and carageenans as gelling agents for NPV production[1]

Gelling agent	Percent concentration	PIB's per larva ($\times 10^9$)	Comparison to standard
Agar	2	4.661	1.00
Gelcarin	1	4.145	.89
HWG	2	5.421	1.16
Gelcarin	1	4.559	.98
GH	2	3.346	.75
Seagel	1	5.721	1.23
GH	2	6.566	1.41
Seakem	1	3.872	.83
202	2	5.782	1.24

[1]High wheat germ diet; larvae infected in fifth instar.

Table 6.5-27.—Relationship between larval density and NPV yield[1]

Larvae per container	Container volume (mm³)	PIB's per larva
1	50	2.19×10^9
5	180	1.76×10^9
10	180	1.58×10^9
15	180	1.37×10^9

[1]Larvae inoculated in fourth instar.

Table 6.5-28—Relationship between host density and NPV yield, utilizing fifth-instar female larvae[1]

Larval density	Mean larval weight (mg)	Mean PIB's per larva ($\times 10^9$)	Total PIB's per cup ($\times 10^{10}$)
5	2,110	6.056	3.028
10	1,550	3.581	3.059
15	1,297	2.230	3.345

[1]Modified hornworm diet.

the harvested virus are maximal. In practice, virus-killed larvae are usually harvested (Chauthani and Claussen 1968, Lewis 1971, Smith et al. 1976) because it is assumed that virus yield is greatest. Lewis (1971) removed infected gypsy moth larvae 1 day prior to death and placed them in clean plastic containers under refrigeration, to prevent wilting of the larvae on diet, with subsequent loss of recoverable virus.

In a standard NPV production schedule, larvae were harvested at day 10 postinoculation, the time of about 30 percent mortality. At 29° C, the slope of the mortality curve was quite steep, especially between day 10 (30 percent kill) and day 12 (75–80) percent kill). By day 10, infected larvae have nearly ceased feeding, and virus yield is similar at day 10 to that on subsequent days (table 6.5–29), despite an increase in mortality during the experimental period. Day 10 harvest was chosen in order to minimize wilting of virus-killed larvae and loss of recoverable virus, and to minimize the bacterial load per larva.

At present, a possible "maturation" effect is being investigated; as virus-induced mortality increases, the activity per PIB increases. Preliminary data indicate that viral activity increases (up to eightfold) as the incidence on NPV-induced mortality increases from 0 to 100 percent. Further testing, however, is needed to confirm this observation. Ignoffo and Shapiro (1978) demonstrated a maturation effect of *Heliothis zea* NPV. These differences in activities of virus from living-infected and virus-killed insects were sixfold to twelvefold.

In vivo virus production is simple and straight forward up to the time of harvest. Insects are exposed to virus and become infected. Eventually these insects will die if left undisturbed. The problem arises at this point as to how to maximize virus harvest by minimizing tissue histolysis and subsequent loss of virus by leakage into the diet. Larvae and diet could be harvested or the loss of virus into the diet could be prevented. The former method may seem desirable, but prior virus productions have centered around larval collection.

Ignoffo (1966) and coworkers collected *H. zea* larvae, which were placed in 240-ml containers and stored at -20° C until processing. Virus-killed *Orgyia pseudotsugata* larvae were freeze dried and placed under vaccum in sealed glass containers (Chauthani and Claussen 1968). Smith et al. (1976) collected *L. dispar* cadavers by vaccum.

In the study production run, both living-infected and virus-killed insects were frozen at day 10. Freezing prior to harvest was an advantageous and convenient method for inhibiting bacterial growth within insects, removing the larvae from the containers and removing wilted insects. Larvae, within containers, were placed in a large freezer (-20° C) and held overnight. The following day, the frozen larvae (including those wilted) were easily removed by spoon or forceps. Four persons could harvest 10,000 larvae in 1 hour.

Processing

Standardized methods should be developed with these goals in mind: Recovery of virus from infected or killed insects should be maximized; activity of virus should be preserved; and contaminant levels should be minimized.

Preprocessing Storage

Ignoffo (1966) suggested that only living-infected or recently killed larvae should be used for processing. Infected gypsy moth larvae were refrigerated and allowed to die (Lewis 1971). In other instances, cadavers were processed after harvest (Smith et al. 1976). In the program study production scheme, larvae were placed in plastic bags and frozen until processed.

Table 6.5–29.—*Incidence of NPV-induced mortality and yield in relation to time of harvest after inoculation*[1]

Days after inoculation	Percent mortality	PIB's per larva
6	0	1.30×10^8
8	1.4	1.05×10^9
10	31.7	1.75×10^9
12	81.9	2.07×10^9
14	97.8	1.53×10^9
16	100.0	2.07×10^9

[1] Larvae infected in fourth instar.

Methods

Methods of recovery are quite diverse, but in general involve extraction of virus from the insect, filtration, and concentration. After concentration, virus may be stored as suspensions or powders under refrigeration, freezing, or ambient conditions (Ignoffo 1966).

Recovery Techniques

The next step in the production scheme is of crucial importance—the recovery of as much virus as possible from the virus-infected and/or virus-killed insects. The classical method has been to blend the larvae in water and then filter the material through cheesecloth and/or nylon (Ignoffo 1966, Lewis 1971, Smith et al. 1976).

Surprisingly, little attention has been paid to the blending process itself. For all our processing, the standard procedure is to blend 1 g of larval weight (frozen) in 10 ml of water for 60 seconds. Following filtration through coarse cheesecloth, about 95 percent of the PIB's can be recovered from infected larvae. The relationship between dilution (gram larval weight per milliliter water) and PIB recovery was investigated. At dilutions of 1:1 to 1:5, less than 40 percent of all PIB's were recovered after a single blend. At a dilution of 1:10, however, recovery was increased to 94 percent. At greater dilutions, recovery was only increased slightly.

Blending time can be very important, as inadequate blending will result in low PIB yields. At a larval weight:water dilution of 1:10, a 5-second blend resulted in a recovery of 75 percent of the total available PIB's. When the blend time was increased to 15 seconds, recovery was increased to 98 percent. Additional blending time resulted in little additional recovery.

Concentration of Product

In general, centrifugation has been used to concentrate the PIB suspension prior to drying. Ignoffo (1966) centrifuged bollworm virus at 5,000–15,000 rpm for 15 minutes in order to ensure a high recovery. Smith et al. (1976) allowed PIB's to settle for 1 week under refrigeration, prior to continuous flow centrifugation (9,000 rpm). Lewis (1971), on the other hand, collected gypsy moth PIB's by differential centrifugation.

Centrifugation, if it is to be economical, cannot be differential but must be carried out in a single run. At the Otis laboratory, virus was centrifuged at about 7,000 rpm for 20 minutes. Thereafter, the pellets were removed and air dried.

The question arises as to whether or not centrifugation is necessary to obtain the desired virus product. Initial tests involving elimination of the centrifugation step indicate that a suitable virus product may be obtained by direct air drying of the filtrate. Thus, the total PIB concentration and bacterial contaminant levels were similar in air-dried pellets and the air-dried filtrates. A problem that arises in the standard production of blending, centrifugation, and air-drying is the removal of large volumes of water. Water is removed via centrifugation, which requires either a continuous-flow system or a large-volume centrifuge. To this end, a great deal of effort is being made to utilize freeze drying as a feasible system. In this system, infected larvae are harvested, freeze dried, and dehaired. Following dehairing, the larvae are dry blended and sieved. The resultant powder should contain a minimal amount of urticarious and allergenic setae and a maximal amount of virus. Preliminary tests indicate that the powder contains about 1×10^{10} PIB's per gram.

Storage of Processed Virus

The goal of this research is not only to maximize virus yield per larva and to produce highly active virus, but also to minimize the bacterial load per larva. From a microbiological point of view, the bacterial population per larva should increase as the number of virus-killed larvae increases. Data from fig. 6.5-19 indicate that this premise is true. It should be noted that these counts were made prior to processing and drying and that they represent the inherent bacterial "loads."

Most of the work on processing and storage of the gypsy moth NPV involved both air-dried and freeze-dried powders, which will be tested for storage stability under standardized conditions.

Quality Control

The aim of production is to obtain virus that conforms to certain specifications. The product should be characterized on the basis of biological activity, bacterial contaminant levels, and safety. Our laboratory is involved routinely with activity and contaminant levels. The intent of this chapter is not to discuss quality control in detail (see NPV Production Quality and Control in chapter 6.3) but to analyze certain aspects of this important area.

Activity

Activity of NPV is measured by bioassay against gypsy moth larvae, under standardized conditions. At the present time, however, each investigator sets his own standards (Doane 1969, Magnoler 1970*b* and 1974) concerning virus inoculum, test insect, diet, temperature, etc. Within the past year, however, some progress has been made on standardization, as the New Jersey colonized strain of *L. dispar* is now being utilized by other laboratories.

The Hamden isolate of gypsy moth NPV has been utilized for in vivo virus production because of its high activity (Magnoler 1974). Some effort, however, should be made to survey and evaluate other geographical isolates in terms of activity and yield against the standard insect. It is hoped that other parameters such as diet, temperature, container host, density, and length of bioassay period will become standardized.

Since virus yield per larva may become standardized, its measurement should be part of quality control. Although variation in yield occurs from larva to larva, acceptable minima can be established. Utilization of female larvae for virus production has both increased virus yields and decreased variations due to inherent sexual differences between males and females.

Determination and Minimization of Exogenous Contaminants

Each production lot should be monitored to guarantee that the types and numbers of microbial contaminants meet established standards (Ignoffo and Shapiro 1978). Total contaminant levels reflect both preharvest and postdrying levels.

Preprocessing Levels

Several workers (Cline et al. 1972, Ignoffo and Heimpel 1965, Ignoffo and Shapiro 1978, Podgwaite and Cosenza 1966) showed that contaminants, in numbers and types, are greater in virus-killed larvae than in living-infected larvae. Program data (fig. 6.5–19) indicate that the bacterial population (=total
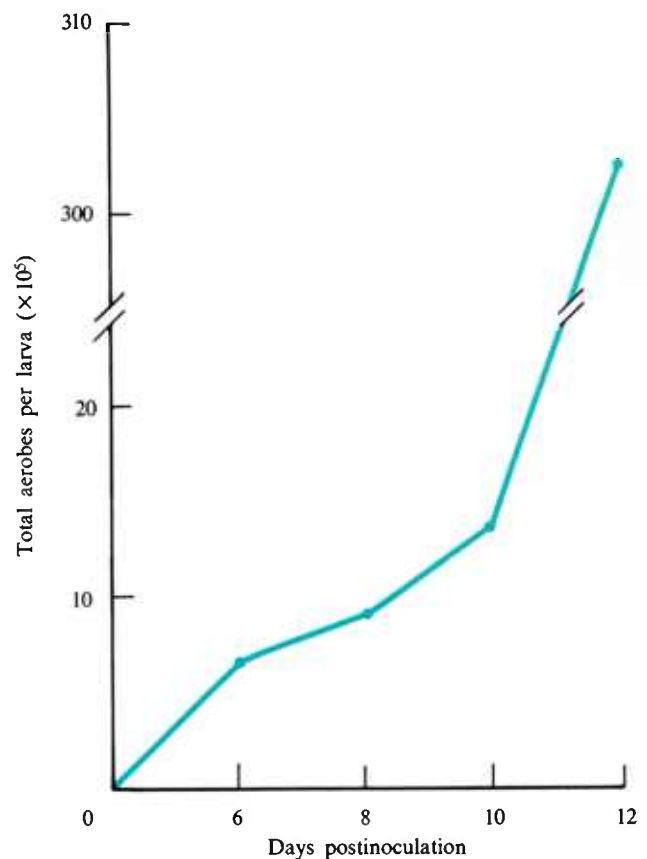
Figure 6.5–19.—*Bacterial loads in gypsy moth larvae during the course of nucleopolyhedrosis.*

aerobes per larva) increases during the infection cycle. Thus, the inherent, preprocessing bacterial load can be minimized or maximized, depending upon the original contaminant level (fig. 6.5-20).

It may be important to determine the bacterial levels in the water used in blending. Unfortunately, significant differences may exist, which would contribute to the predrying bacterial load. In a series of tests, tap, distilled, and sterile distilled water were compared as diluents. Data indicate that bacterial levels in the tap water were quite variable and sometimes high. Distilled water levels were sometimes high but only after prolonged storage in the holding tank. Levels from larvae blended in sterile (autoclaved) distilled water were lowest. For routine processing, distilled water was used as the diluent.

Postprocessing Levels

At present, the gypsy moth NPV is specified as an air-dried powder. This method, however, must be controlled carefully, in order to minimize the buildup of microbial populations during drying. As the volume of virus suspensions increases, the drying time and the final bacterial load in the product increase (table 6.5-30). In general, the inherent bacterial levels (day 10 harvest) varied between $3-5 \times 10^6$ aerobes per gram. As the volume increased from 100 to 500 ml, the bacterial populations increased logarithmically.

Methods of Drying

Horizontal vs. Vertical Hoods

During the pilot production run, the NPV was dried in a clean air vertical laminar flow hood under prevailing ambient conditions. In a series of tests, the efficiency of a horizontal laminar flow cabinet (41 cm L×33 cm W×28 cm H) equipped with a dehumidifier was compared with a vertical laminar flow hood (185 cm L×65 cm W×71 cm H) under ambient conditions. Both were equipped with 99.97 percent high efficiency particulate air (HEPA) filters.

As in previous tests, the inherent, preprocessing bacterial loads (from larvae harvested at day 10) were quite low, about 3×10^7 aerobes per gram. The bacterial level was higher after drying in the vertical hood (fiftyfold increase) than in the horizontal

Figure 6.5-20—*Relationship of initial and final bacterial load in an air-dried product (aerobes per 200-ml sample).*

Table 6.5-30.—*Bacterial counts in relation to volume of NPV suspension predrying and postdrying*[1]

Volume of NPV suspension (ml)	Total aerobes per gram (× 10⁶)	
	Predrying	Postdrying
100	4.5	10.1
250	4.0	87.8
500	4.7	172.8

[1]NPV harvested from larvae 10 days postinoculation.

chamber (tenfold increase). In general, greater contaminant levels resulted from slower drying under ambient conditions. In all subsequent tests, the horizontal flow chamber was used.

Dehumidification

The horizontal flow chamber was adapted so that the chamber could be dehumidified with a BRY® air dehumidifier. During full dehumidification, the temperature in the chamber increased from 25° to 38° C, while the relative humidity decreased from 40 to 0 percent. When the dehumidifier was on 50 percent of the time, the temperature increased to 32° C, while the relative humidity decreased to 20 percent. The product can be dried much faster in dehumidified air, thus avoiding the buildup of bacteria that occurs with the conventional air drying. When air-dried virus was bioassayed, no differences in activity were observed following dehumidification.

Freeze Drying vs. Air Drying

In the previous section, it was shown that air drying did not inhibit bacterial multiplication. Whether the virus suspensions were centrifuged or not, the bacterial levels were similar in viral pellets and filtrates. Moreover, if initial bacterial levels were high, from virus-killed insects, the final bacterial levels could exceed the acceptable limit of 10^9 aerobes per gram. Because of these concerns, tests were initiated to compare air drying with freeze drying. In these tests, as in previous air drying tests, frozen infected larvae (day 10 harvest) were blended in sterile water. Bacterial counts were taken of the filtrates before and after drying.

Freeze drying resulted in a decrease in total aerobes per gram from 4.6×10^7 to 3.2×10^7. Air drying, on the other hand, led to a sixteenfold increase from 4.9×10^7 to 77×10^7 per gram. From these experiments, it was demonstrated that the total aerobes per gram can be minimized by a combination of harvesting insects at a time when mortality is low (day 10); freezing the infected and/or dead insects, thereby preventing further bacterial multiplication; and freeze drying the

viral suspension, resulting in a reduction of aerobes per gram.

Prototype Facility for Mass Production of Virus

Basic Design

Structure

The prototype facility was designed as a quarantine facility, having the capacity to rear and process 12,000 larvae per day (fig. 6.5-21). The facility consists of a rearing room (3.6×5.5 m), which is a modular environmental chamber. The main part of the prototype (46 m²) is a work area where virus inoculation, harvest, and processing can be done. After harvest, the infected larvae are held in a freezer (2.5×3.6 m) that has the capacity of storing several days harvest as well as the processed virus. The entrance to the facility is via a clean-air shower, which workers must pass through. A 2.5×3 m room is provided, which may be utilized as an office or laboratory. Adjacent to the office is a room equipped with a steam cleaner, where all carts and trays leaving the area will be cleaned. Restrooms for men and women were included for worker cleanup before leaving. Except for the restrooms, the entire facility is comprised of modular panels from the former mass-rearing facility.

Environmental Control

The rearing room has its own temperature and humidity control equipment, in addition to a separate air-circulating system equipped with a 99.97 percent HEPA filter to clean the air in the chamber. The work area and office have an air-conditioning and heating system that recirculates the air every 3 minutes through a 99 percent HEPA filter.

Contaminant Control and Quarantine Considerations

To reduce the possibility of virus-laden air entering other parts of the mass-rearing facility, the rearing and work areas are under negative pressure. A small fan draws air out of the rooms through a 99.95 percent

A. Air shower with hepa filter

B. Men's restroom

C. Women's restroom

D. Office

E. Sanitizing trays and carts

F. Area for inoculation of virus and harvest of frozen infected larvae

G. Virus processing area

H. Walk-in freezer for freezing infected larvae and virus storage

I. Environmental chamber for incubation of virus-inoculated larvae
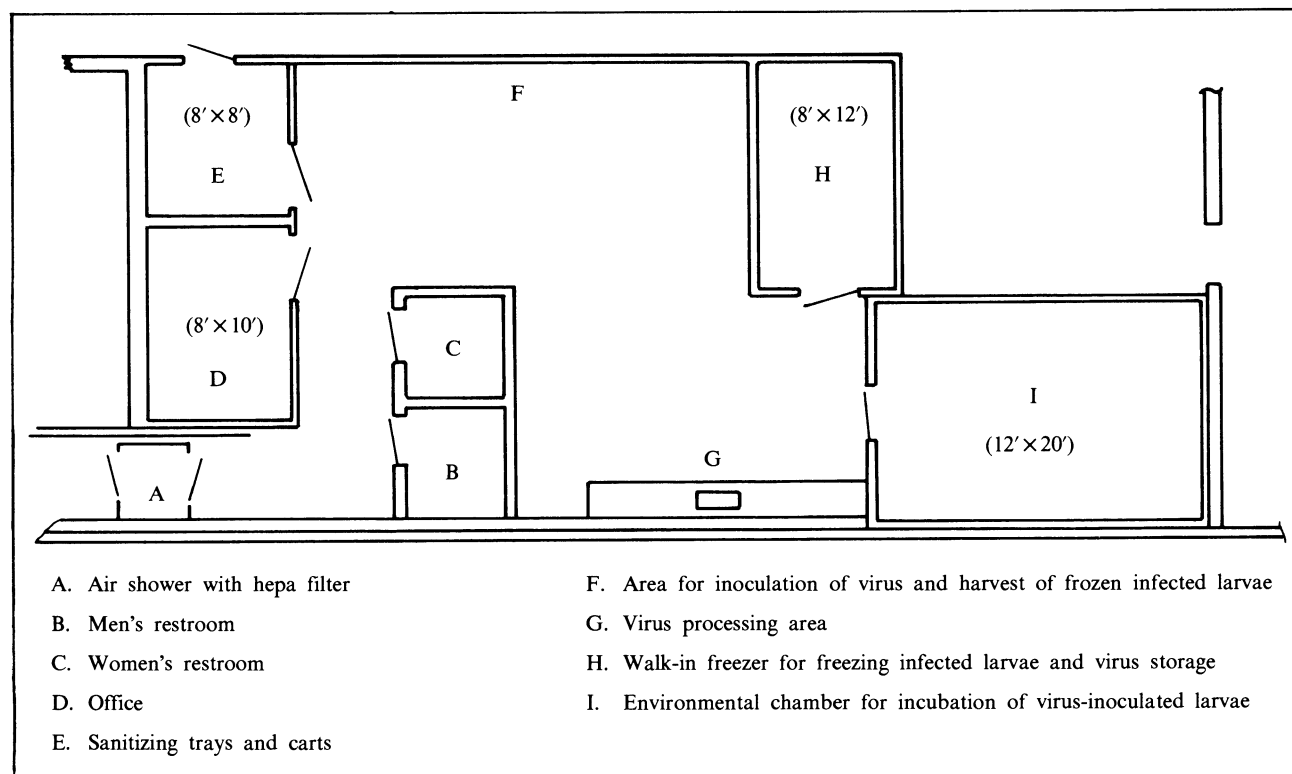
Figure 6.5-21.—*Prototype production facility for gypsy moth virus.*

HEPA filter at a rate of 4.2 m³ per minute, before blowing it outdoors. The air shower should reduce the amount of contaminants carried on personnel leaving or entering the facility; high velocity, sterile, low-pressure air (368 m per minute) collects contaminants from the workers. The air-borne particles are exhausted through floor level ducts and returned to the blower section for recycling through 99.97 percent HEPA filters.

The area for infecting larvae and processing virus has a hood system over the entire work counter. The air drawn into the hood is passed through HEPA filters and returned to the room, thus minimizing contamination from spraying of virus or virus processing.

Equipment and Operation

Diet making, filling of cups, and insect rearing (up to the fifth instar) are performed in the main (separate) rearing facility. Therefore, each day a cart 0.68×1.2 m) holding up to 12,000 larvae and cart with fresh diet will enter the virus facility through the air shower. Also, two carts will leave through the steam room, one coming from virus production and one that held the incoming larval diet. All other operations of virús production can be carried out in this facility, from infestation to virus processing and storage.

Virus Inoculation

Since the larvae tend to feed primarily around the edges of the containers, the heaviest application of virus should be on the outer edge. A pipet injects the correct volume each time into the nozzle. Air is used as the atomization medium. A solenoid can be used to activate the pipet. A switch or timer activates the solenoid, depending on whether the cups are handled by hand or whether a conveyor is used to move them under the sprayer. After the excess moisture has

evaporated, the insects are transferred onto the virus-treated diets by hand. Handling of the insects is simplified by infesting the same number of insects in rearing and in virus production, so that the insects do not have to be counted but are merely brushed from one container to the other. Utilizing the same type of containers for rearing and virus production saves time and labor, as most larvae tend to "rest" on the paper lids and are thus easily transferred.

Work Area

A special work table, a clean air hood mounted on wheels, was constructed for the protection of the workers during the transfer and harvest of pupae. The tabletop is a 64 cm wide counter with long slots cut into the splash board. Plexiglass sloping down to the splash board is used as a hood. Lights are mounted over the plexiglass to improve visibility. The slots are ducted to a fan below the counter. The air is pulled into the slots at about 150 m per minute, to draw setae, hairs, and scales away from the workers.

Harvest

At 10 days postinoculation, the virus infected larvae are frozen (within their containers) in a walk-in deep freezer (2.5×3.6 cm). The freezer was installed so that carts coming from virus rearing could be placed in the chamber (–20° C). Frozen larvae, including wilted ones, are easily harvested by workers. During the pilot production effort, 10,000 larvae were harvested manually in 1 hour by four workers (40 insects per minute per worker). The freezer is used both for storage of harvested larvae and processed virus.

Processing and Storage

Lyophilization of intact, infected larvae followed by dehairing, blending, and sieving appear to be efficient procedures for processing virus. The larva has to be dried before it can be dehaired. Several methods have been tried, but at the present time, freeze drying appears to be the most promising. Much effort was made to evaluate air drying. HEPA filters were used on the incoming air (80 percent) and on the exhaust side (99 percent). A dry-air dehumidifier and

a room air-conditioner were placed on the system in various combinations. Air drying of virus suspensions, following blending and filtration, can be accomplished without too much bacterial buildup or too high a temperature (that might inactivate the virus) by bringing room air into the dehumidifier and through an air-conditioner before entering the chamber. Frozen, infected insects cannot be used in this procedure, however, as the insects thaw and wilt before drying.

Dehairing is accomplished by placing the freeze-dried insects into a plastic screen (6 mesh) cylinder and shaking for 1 or 2 minutes. The setae are easily broken and pass through the screening, where they are collected by vacuum. The dehaired larvae are then dry blended (1 minute), and the resultant material is passed through a series of sieves. Thus, the technical virus powder can be standardized to particle size. The virus powder will be stored in the freezer, until storage stability studies are completed.

Scheme of Operations

The virus production system involves several operations: Virus inoculation, larval transfer to inoculated diet, rearing of infected larvae, lyophilization of intact, infected larvae, dehairing of freeze-dried larvae, and blending, sieving, and storage of virus powder. All processes can be performed in the virus prototype facility. Virus inoculation and processing (dehairing, blending, sieving) take place at the work hood area. Larval transfer and harvest are carried out at the clean-air work table, thus reducing contamination. Following transfer of fifth-instar female larvae (5 per 180 ml cup) on virus-treated diet, the containers are capped (paper lids), placed on the mobile cart, and transferred to the environmental chamber (29° C, 50 percent relative humidity, 12 hours light/12 hours dark) for 10 days. At the end of the holding period, the cart is taken into the walk-in freezer (–20° C). The following day, the cart is taken into the work area, and larvae are removed for freeze drying. Following lyophilization, the dried larvae are processed, and the technical virus powder is stored in the freezer. All carts and trays are then moved into the steam-cleaning room, where they are cleaned. Equipment (blenders,

forceps, etc.) and used containers are autoclaved. Heat-labile equipment is disinfected (sodium hypochlorite or formalin) to eliminate viral contamination. The facility can be sanitized or steam cleaned at regular intervals.

Production Efficiency

Investigations in several areas are in progress in order to increase production efficiency, particularly in regard to the insect, the container, the diet and processing.

The Insect

The use of fifth-instar female larvae greatly increases virus yields: It has been possible to obtain $5-8 \times 10^9$ PIB's per larva. Thus, 20 larvae or fewer can provide enough virus for 0.4 ha application (1×10^{11} PIB's). If the same group of insects can be selected for sterile male (males) or virus production (females), an efficient use of insects could be realized. After 21 days on diet, the larvae can be sexed. The males are placed on new diet to complete larval development, and subsequent irradiation as pupae, while the females are utilized for virus production.

The Container

At present, optimal growth of the female larvae and maximal virus yield are obtained with five larvae per 180 or 240 ml container. If a suitable rearing container or tray can be developed, costs of containers may be reduced further.

The Diet

The goal of a more simplified diet, using carrageenan as the gelling agent is being researched. Tests indicate that this approach is feasible.

Processing

The new processing methodology, utilizing freeze-dried, intact larvae, should result in a bacteriologically cleaner, less costly technical material that requires fewer procedures and therefore less labor to produce (table 6.5-31).

Table 6.5–31.—*Comparison of operations involved in processing NPV by alternative methods*

Present method	New method
Blend	Freeze dry
Cheesecloth filter	Dehair
Reblend	Blend (dry)
Refilter	Sieve
Centrifuge	Grind
Air dry	
Grind	

Costs

The economics of in vivo NPV production are a combination of costs of production of larvae prior to virus inoculation, costs involved from inoculation to harvest, and operations from processing and storage.

In addition, quality control and safety testing must also be considered as necessary and vital adjuncts to virus production. The estimated cost of *Heliothis* NPV production, excluding overhead, was calculated at 7 cents per larva or 1 cent per billion PIB's. Labor costs were higher than both rearing containers and diet combined (Ignoffo 1966). It was felt that costs could be reduced through more efficient rearing techniques (automation of egg collection and larval infest) and virus production techniques (automated virus inoculation). Further cost reductions could be realized by optimization of containers and diet.

Smith et al. (1976), utilizing third-instar *L. dispar* larvae, produced NPV at the cost of 8 cents per larva or 10 cents per 10^9 PIB's. During the pilot production run, NPV was produced (2 cents per larva) and processed (about 1.5 cents per larva) for about 3.5 cents per 10^9 PIB's. From these data, it is evident that gypsy moth NPV production became more efficient and less costly because of more efficient mass-production technology and more efficient virus production technology. The use of fifth-instar female larva (2 cents per larva, or about 0.5 cent per 10^9 PIB's) plus new processing procedures (about 1 cent per larva) should result in a considerably greater reduction in cost.

In summary, research in both mass-rearing technology and NPV production has resulted in a

more efficient system. The use of mechanization or automation in both rearing and NPV production should result in even greater efficiency. To this end, the judicious use of mechanization is being investigated where feasible.

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6.6 Phytochemicals and Feeding Behavior of Gypsy Moth Larvae

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Introduction

It has been recognized for some time that the selection or rejection of a plant as a food source by an insect is determined mainly by chemical composition of the plant. That the nutritional aspects are not of primary importance became evident as data accumulated on the nutritional requirements of various insects. In general, these requirements were little different from those of other animals with a need for vitamins, essential amino acids, certain lipids, minerals, and carbohydrates as energy source. In addition, insects need a sterol as a unique supplement, because they lack a necessary enzyme in the general pathway for steroid biosynthesis. A recent review summarizing the nutritional and metabolic aspects of insect-plant interactions was made by Beck and Reese (1975). Since the composition of leaves is not much different qualitatively among species for the primary metabolites (those present in all living matter such as amino acids, fatty acids, nucleotides, simple carbohydrates, vitamins, etc.), attention was directed towards the so-called secondary metabolites as they function in the herbivore selection process. Fraenkel (1959) described the general relationship between these substances and phytophagous insects based on the evidence available at that time. A more recent general view of chemical inhibition of feeding of phytophagous insects is by Chapman (1974).

Secondary metabolites, which include glycosides, terpenes, flavonoids, alkaloids, etc., are less widespread and are generally restricted to families, subfamilies, and even genera. As a result, their influence on insect behavior by serving as attractants and repellents (deterrents) places them in a primary position of host-plant interactions. A decade later Fraenkel (1969) updated his original ideas to include a broader function for these substances with a role in insect physiology and development. Indeed, chemical communication is the basis for interaction between species, be it microorganism-plant, animal-plant, plant-plant, animal-animal, and so on (Whittaker and

Feeny 1971), and is the heart of the discipline aptly named chemical ecology.

Current terminology utilizes allelochemics (Whittaker 1970) in place of secondary metabolites for nonnutritional substances of one species that affect the growth, health, behavior, or population biology of another species. If the allelochemic is of adaptive value to the releasing organism, it is called an allomone, and if the advantage is to the receiving species, a kairomone. The underlying principles controlling interspecies relationships seem well established; the job at hand is to utilize this information for practical ends. This section is a summary to date of an attempt to gain information about the specific natural products that control the feeding behavior of the gypsy moth in the larval stages.

The first observations of gypsy moth feeding behavior were made from field studies. Many of these field observations were recorded by Forbush and Fernald (1986) in their classical compendium, along with results of tests with nearly full-grown larvae confined to jars containing known foliage. Of the 477 species of trees, shrubs, and herbs examined, most (458) were consumed to a greater or lesser degree, with only 19 rejected. A more extensive and thorough study, but with fewer species (152), was made by Mosher (1915), in which larvae of each instar were exposed to fresh leaves in trays and reared, if possible, to adulthood. In many cases fecundity was also determined. The plants were then grouped as a food source into four categories: Favored at all stages, favored after early larval stages, not particularly favored, and unfavored. The last two groups contained, respectively, 32 and 63 examples. Clearly, there are many plants that are not preferred hosts for the gypsy moth larvae.

This study began by examining members of this group as possible sources of antifeeding substances, which could eventually lead to a control measure. That naturally occurring feeding deterrents exist for the gypsy moth was demonstrated by Adlung (1957) by spraying acceptable foliage (oak and hazel) with extracts from nonhost leaves (black alder or horse chestnut). This rendered them unacceptable. Furthermore, when lightly fed-on leaves, as those of lettuce or

dandelion, were treated with dilute solutions of sucrose or fructose, an increase in consumption resulted. These experiments suggested the presence of feeding stimulants and deterrents as important factors in regulating gustatory activity. The chemical nature of these materials remained unanswered, however.

Bioassay and Plant Screening

Before work on the isolation and structure elucidation of natural constituents that influence larval feeding behavior could begin, a simple and reliable bioassay had to be devised. In its development the following important points required consideration: A year-round test was necessary for continuous assaying of fractionated material; results should be available in several days; it should be relatively inexpensive; and the results should reflect field observations. Rearing of insects on an artificial diet from field-collected eggs was already possible (ODell and Rollinson 1966), so only the problem of providing them in quantity and at the desired instar remained. Since a prolonged cold treatment of eggs to break the diapause is necessary, a realistic projection of need was required in advance for efficient utilization of resources.

For the bioassay results to be recorded in as quantitative a manner as possible, an end point based on measurement of mass would be desirable. Consequently, either the amount of diet consumed or fecal matter produced could be used; the latter was chosen because it required less handling to get a constant dry-weight value. After earlier attempts with a diet of powdered dried leaves were unsatisfactory, success was obtained with a regimen consisting of an alcoholic leaf-extract residue incorporated into a simple aqueous cellulose-agar media (Doskotch et al. 1977a). By this method, in which the diet is an approximation to a reconstituted leaf, the extractables from different sources can be compared, on an equal weight basis; the nonextractables (polysaccharides, lignins, etc.), which vary widely among leaves, are eliminated. The incorporation of oak leaf extract into an aqueous cellulose-agar base produces a readily acceptable preparation that can be manipulated either

by varying the amount of extract or by admixing other plant extracts with the basal media. A dose response curve resulted when the quantity of red oak extract was altered. However, the response reached a maximum when 1.0 gm of residue was incorporated into about 50 ml of diet. Additional residue did not increase the amount of feeding. The 1.0 gm level was chosen for the feeding control or standard diet. Red oak (Quercus rubra L.) was selected as the standard. Leaves collected during the time the larvae are active were air-dried in the shade, mechanically powdered, and stored. Extraction was made by percolation with ethanol at room temperature; the ethanol was removed by evaporation at reduced pressure and at a temperature no higher than 40° C. A 3-month supply of the residue is readily prepared in this manner and can be stored in the refrigerator for extended periods.

A screening procedure for plants containing antifeeding substances was developed on the basis of the success with the control diet. A sample (0.25 gm) of the test-plant residue, prepared in the same manner as the oak residue, was incorporated into the oak control diet. One gram of the test-plant residue was also mixed with the aqueous cellulose-agar media. Thus three separate diets constituted a complete test: An oak control, a test plant plus oak mixture, and a test plant alone. One hundred newly emerged third-instar larvae divided into groups of 10 were exposed to each diet. After 24 hours the fecal matter was dried to constant weight and served as a direct measure of the amount of diet consumed. The frass produced on a control diet ranged between 30 to 60 mg for 10 insects; an average of the 10 replicates was the recorded value. Selection of third-instar larvae (a compromise) provided the advantage of sufficient amount of frass for quantitation, with retention of adequate food discrimination sensitivity. In addition, third-instar larvae are generally more uniform in the time it takes to molt from the previous instar than are other instars, and their size facilitates transfer to and from the test dishes.

The three-diet screening technique indicated four types of feeding responses. These were designated as stimulant, neutral, deterrent, and synergistic, hence making it possible to group plants into these cate-

Table 6.6-1.—*North American plants screened in feeding test for larvae of Lymantria dispar L. and classified as deterrent*[1]

Common name	Latin name	Family	Origin	Collection period	Percent feeding
Trees, forest					
Blackgum	*Nyssa sylvatica* Marsh	Nyssaceae	N.C.	7/73	48
Black walnut	*Juglans nigra* L.	Juglandaceae	Ohio	7/73	32
Catalpa	*Catalpa speciosa* Warder	Bignoniaceae	Ohio	8/73	29
Eastern red cedar	*Juniperus virginiana* L.	Pinaceae	N.C.	7/73	14
Hop-tree	*Ptelea trifoliata* L.	Rutaceae	Ohio	7/74	18
Red maple	*Acer rubrum* L.	Aceraceae	Conn.	8/72	49
Tuliptree	*Liriodendron tulipifera* L.	Magnoliaceae	Mass.	5/73	53
Trees, ornamental					
Cajeput	*Melaleuca leucadendra* L.	Myrtaceae	Fla.	5/73	44
California buckeye	*Aesculus californica* (Spach) Nutt	Hippocastanaceae	Calif.	3/74	51
California-laurel	*Umbellularia californica* Nutt	Lauraceae	Calif.	1/74	32
Shrubs					
Great laurel	*Rhododendron maximum* L.	Ericaceae	Penn.	8/73	26
Japanese andromeda	*Pieris japonica* D. Don	Ericaceae	Penn.	Unknown	14
Japanese honeysuckle	*Lonicera japonica* Thunb.	Caprifoliaceae	Conn.	8/72	19
Mountain laurel	*Kalmia latifolia* L.	Ericaceae	Mass.	8/72	6
Sweet pepperbush	*Clethra alnifolia* L.	Clethraceae	Conn.	7/72	55
Ferns					
Cinnamon fern	*Osmunda cinnamonea* L.	Osmundaceae	Ohio	7/73	45
Common polypody	*Polypodium vulgare* L.	Polypodiaceae	Ohio	7/72	14 (rhizomes)
Hayscented fern	*Dennstaedtia punctilobula* Moore	Polypodiaceae	N.J.	8/73	21 (tops)
New York fern	*Thelypteris noveboracensis* (L.) Nieuwl.	Polypodiaceae	Ohio	7/73	29

[1]A deterrent plant showed feeding less than 60 percent in combination with the control (oak) extract.

gories. A stimulant extract gave good feeding when tested alone or in combination with oak. A neutral extract produced little feeding alone but did not alter the active feeding when admixed with oak. A deterrent extract showed low feeding alone or when tested together with oak. The quantity of fecal material of the control was taken as 100 percent, to which the other diet results were compared. A synergistic extract showed low feeding alone but in combination with oak caused a severalfold increase.

Results from foliage testing of 190 North American species or varieties uncovered 30 stimulant, 82 neutral, 57 deterrent, and 8 synergistic sources. The remainder (13) had variable results. Thirty examples of these are given by Doskotch et al. (1977a). Table 6.6-1 lists some of the more common plants classified as

deterrent. Since the screening procedure was instituted primarily for indicating sources of antifeeding substances, little attention was paid to the season of collection. Therefore, extrapolation of the results to predict susceptibility to attack under field conditions must be guarded. The data can be valid if the collection time and larval presence coincide, because plant constituents are known to vary with foliar age. A clear illustration occurred with September collections of eight species of southern oaks from Georgia; these were not stimulatory but were either neutral or deterrent. A recollection made in June gave leaves with mainly stimulant properties. It is already documented (Feeny and Bostock 1968) that tannins in oak leaves exhibit seasonal variation, ranging from 0.5 percent dry weight in April to about

5.0 percent in September. Tannins are recognized as general precipitating agents for proteins and at high concentrations disrupt the protein-mediated digestive system. Their presence explains the deterrent results with some of the southern oaks. The neutral results may be due to loss of stimulatory constituents and lower levels of tannins.

A number of conclusions can be drawn from the screening results. First, larval feeding is a chemically initiated response and any potential host must produce them. Second, field-observed nonhosts are plants that lack stimulants or contain either deterrent or synergistic constituents; differentiation among the three is only possible through bioassay. Third, the synergistic response (an unexpected result) suggests a multifaceted mechanism for feeding stimulation.

Fractions derived from partitioning of the deterrent extract among four immiscible solvent pairs (see section on Isolation Methods) were added to the base diet in the same manner as the crude extract. Subsequent fractions, obtained through high resolution techniques, required the dilution and/or suspension of relatively minute quantities in a solvent. Such solvents must give a uniform distribution of the fraction when admixed with the red oak agar base and must not significantly affect the larval feeding response. Within these constraints, distilled H_2O, 95 percent ethyl alcohol (EtOH), and N,N-Dimethylformamide (DMF) were found to be acceptable carrier solvents. Methocel type MC, in EtOH was used as a suspending agent when a fraction did not readily dissolve in a carrier solvent. Dose response curves resulted when the quantity of each respective carrier or suspending agent was altered in the red oak control diet. Satisfactory results were obtained when doses did not exceed 1 ml EtOH, 1 ml DMF, and 2 percent Methocel in 1 ml EtOH. When tests required carrier solvents and/or suspending agents, additional controls, with like quantities, were run concurrently.

Modification of the test procedure was implemented when a batch of the crude red oak control extract failed to cause feeding above 25 mg frass weight equivalent for 10 insects. Previous tests by Godwin determined that 0.1 M sucrose added to a cellulose-agar base caused feeding of the gypsy

moth. Using this information, a 0.1 M sucrose solution (40 ml) was substituted for the 40 ml of H_2O normally used in the aqueous agar-cellulose base and bioassayed with the red oak control extract to determine the effect of the sucrose solution on larval feeding. In addition, a previously tested deterrent extract was admixed with the red oak sucrose base to determine if the sucrose altered the level of deterrency, as compared to previous tests without sucrose. The addition of sucrose increased feeding by approximately 50 percent and did not change the relative level of deterrency; that is, if a deterrent fraction had previously decreased feeding by 60 percent, that level of deterrency was maintained in tests utilizing 0.1 M sucrose as a feeding stimulant. Subsequently, a 0.1 M sucrose solution was used to enhance test sensitivity whenever a particular batch of red oak control extract failed to provide an adequate feeding response ($\geqslant 25$ mg frass weight equivalent).

The incidence of inadequate control feeding appears to be related to the use of control extracts originating from leaf collections from specific localities, but the site, plant, and insect (gypsy moth larva) interactions causing the reduced feeding have not been determined.

Test sensitivity was also enhanced by extending the feeding time. A 42-hour test period, beginning around 1600 hours, was compared with the former 24-hour period to determine relative levels of feeding on control and test diets. Feeding during the 42-hour test period was proportionate to the increase in feeding time, and the relative feeding response to deterrent fractions was maintained. The maintenance of the deterrent response over an extended feeding period is indicative of the stability of the chemosensory interaction between the plant (active fraction) and the insect; this indicates the relative sensitivity of the bioassay procedure.

The inclusion of the 42-hour test period in the bioassay procedure also solved a logistical problem. The initial procedure required the newly eclosed third-instar larvae to be placed with the test diet before 1300 hours, in order to give larvae sufficient time to construct feeding mats before the nocturnal feeding period began. As the number of related fractions to be

tested concurrently increased, the feasibility of this time schedule became unrealistic. The change in set up time (1300 to 1600 hours) and extension of the test period solved this problem.

Isolation Methods

A general systematic approach to the isolation of active constituents from the selected plants was based on previous experience in the isolation of biologically active constituents in the laboratory and required the use of mild and reproducible techniques. The total alcoholic extract contained constituents from the least polar lipidlike to the very polar glycoside and phenolic type; thus the initial fractionation involved a series of partition steps with different pairs of immiscible solvents. This sequence, which can easily be scaled up, afforded five fractions varying in polarity. The first step utilized choloroform-soluble and water-soluble fractions. The choloroform-soluble residue was next partitioned between 10 percent aqueous methanol and hexane to give the hexane-soluble and methanol-soluble fractions. The least polar constituents were located in the former and the next polar in the latter. The aqueous phase from the initial partitioning was extracted successively with ethyl acetate and *n*-butanol to give the least polar and the medium-polar components, respectively, of the water solubles; the very polar substances remained in the final aqueous solution. Bioassay of all five fractions located the activity and gave some information as to the subsequent isolation techniques to be tried. Invariably the partition procedure produced some insoluble interfacial solids that were removed either by filtration or centrifugation. These were also bioassayed but generally were not active and did not amount to a significant portion of the total extract.

Calculation of the dose for bioassay was based on the relative activity of the crude residue, but since experience showed that several fractions are often active, a doubling or tripling of the calculated value was required to fully measure the activity distribution. Application of the solvent partitioning procedure to over 30 deterrent extracts revealed that the activity was not restricted to any one fraction. For some plants the activity was present in the more polar fractions, in others in the less polar; for a few it was distributed throughout all of them. The water solubles were the least likely to be deterrent. Although nothing can be stated about the chemical nature of the compounds from this study, it was shown that a variety of substances can be expected.

The next step in the fractionation was to employ a high resolution technique and most commonly adsorption chromatography on silicic acid (also called silica gel). This material is an active adsorbent yet is less prone to cause chemical change of sensitive constituents. Also, it is possible to recover the applied sample nearly quantitatively. The choice of conditions for chromatography, such as eluting solvents and activity of adsorbent, was made from thin-layer chromatographic examination of the crude fraction. During the column separation, the eluted fractions were monitored by dry weight and thin layer chromatographic analysis. In the latter procedure, detection was by absorption of ultraviolet light and by spraying with sulfuric acid—a general spray reagent that reveals a wide range of compounds. Similar column fractions, as revealed by the detecting methods, were pooled and bioassayed. Further purification of the active column fractions required custom development of conditions peculiar to the nature of the components in the fraction. At each step repeated bioassay was performed. Also, every attempt was made to account for the total amount of material separated; nothing was discarded prior to biological testing. In this way it was hoped that loss of any constituent would be minimized and that in the end some statement could be made about the contribution of each to the total activity of the leaves. Once homogeneous components were isolated, standard physical and chemical methods were applied to elucidate their structures.

Specific Plants Investigated

Following the bioassay leads for deterrent sources, large collections of leaves were obtained and isolation studies initiated. A current summary of results is given under each individual plant heading that follows.

Many more plants were begun, but either loss of activity during fractionation or inability to consistently reproduce the activity required that the studies be abandoned.

Liriodendron tulipifera L.
(Magnoliaceae)

The antifeeding activity of the crude extract was located in the aqueous methanol fraction after solvent partitioning. A series of adsorption column separations on silicic acid yielded a total of seven new sesquiterpene lactones of which six are illustrated (fig. 6.6-1). All had some measure of deterrent activity, with lipiferolide and epitulipinolide diepoxide being the most abundant. Their characterization has been reported by Doskotch et al. (1975) and was accomplished by relating them to epitulipinolide, the major lactone of the root bark of this species (Doskotch and El-Feraly 1970). Lipiferolide is the 4,5-epoxide of epitulipinolide. Peroxyferolide, a unique natural product, is the first recognized sesquiterpene hydroperoxide and one of three hydroperoxides isolated from natural sources. Its structure, established by physical and chemical methods, was synthesized from lipiferolide by photooxygenation (Doskotch et al. 1977b).

Of the remaining four terpenes, all of which are minor constituents requiring considerable purification, only cyclolipiferolide, dihydrochrysanolide, and 11,13-dehydrolanuginolide have been characterized (Doskotch et al. 1977c). Cyclolipiferolide and 11,13-dehydrolanuginolide were identified by comparison of spectral data with those from known samples. The former was made by acid cyclization of lipiferolide, and spectra of the latter were obtained from one of the authors of the original report (Talapatra et al. 1973). The allylic alcohol group of dihydrochrysanolide was oxidized to the corresponding α,β-unsaturated ketone to give chrysanolide, a constituent of pyrethrum flowers (Doskotch et al. 1971).

Only Terpene H–I, the seventh sesquiterpene lactone, has not been assigned a unique structure. It has the composition $C_{17}H_{22}O_5$, as do four of the terpenes in figure 6.6-1. Besides the unsaturated lactone

functionality, it has a hydroxyl group, an acetate, and two double bonds on a germacranolide skeleton. Paucity of material excluded a chemical solution of the problem, and spectral data suggested equivocal structures. Therefore, an X-ray crystallographic analysis is currently being attempted.

The antifeeding activity (table 6.6-2) of individual sesquiterpenes does not show a direct relationship between dose and response, but approaches a maximum or saturation value. For example, doubling the doses of lipiferolide and epitulipinolide diepoxide causes little change in feeding. The difference between the absolute values is not significant. However, when the two are combined at a dose corresponding to the lowest value for each, a
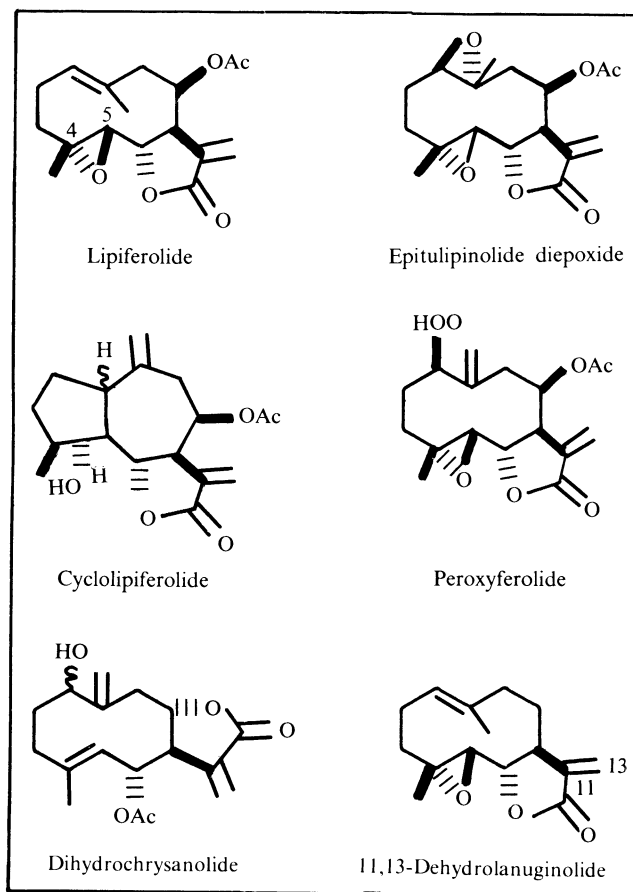
Figure 6.6-1.—*Structures of antifeeding sesquiterpene lactones from* Liriodendron tulipifera *L.*

Table 6.6-2.—*Antifeeding effect of* Liriodendron tulipifera *L. sesquiterpene lactones*

Compound	Dose[1] (μg/ml)	Feeding percent[2]
Lipiferolide	350	62
	750	56
Epitulipinolide diepoxide	350	69
	750	71
Cyclolipiferolide	500	65
Peroxyferolide	250	75
	500	50
Dihydrochrysanolide	110	80
	250	69
11,13-Dehydrolanuginolide	125	79
Terpene H-I	55	69
	250	53
Lipiferolide	375 ⎫	
Epitulipinolide diepoxide	375 ⎭	33
Lipiferolide	375 ⎫	
Epitulipinolide diepoxide	375 ⎬	20
Cyclolipiferolide	375 ⎭	
Lipiferolide	375 ⎫	
Epitulipinolide diepoxide	375 ⎬	24
Peroxyferolide	375 ⎭	

[1]Concentration of compound incorporated into the cellulose agar red oak diet.

[2]Feeding on control diet of red oak was taken as 100 percent.

decided decrease in feeding results. Also, addition of a third sesquiterpene lowers the feeding even further. Apparently, the total effect of the leaf deterrency is due to the combination of all the active compounds, none of which is singularly highly potent. Apparently, each constituent affects a different target, neither of which is dominant in the feeding process. This explains in part the observation made during the fractionation that the crude preparations were consistently more active than the purified components. Furthermore, it is most likely that the crude contains components that themselves are inactive but, by synergistic action, augment the deterrency of the terpenes.

Sesquiterpene α, β-unsaturated γ-lactones have a wide variety of reported activities ranging from cytotoxic, antitumor, allergenic, antimitotic and fungitoxic to schistosomicidal (Rodriquez et al. 1976), and including insect feeding inhibition. For example,

the major lactone, glaucoside A, of several *Vernonia* species is the effective agent in tests with larvae of six Lepidoptera (Burnett et al. 1974). In cases where structure-activity studies were made, the principal contribution to the activity resided with the unsaturated lactone unit, although additional functionalities such as epoxide, hydroxyl, unsaturated ketone, etc., played an enhancing role. One possible mode of action could involve the Micheal-type addition of proteins bearing sulfhydryl groups to the unsaturated lactone (Kupchan et al. 1970). Results would indicate that an additional selectivity is operating beyond the general Micheal-type reaction.

Kalmia latifolia *L.* (*Ericaceae*)

The partitioning sequence when applied to the crude leaf extract of *K. latifolia* distributed the inhibitory activity into three fractions: The methanol solubles, the ethyl acetate solubles (most active), and the *n*-butanol solubles. Column chromatography of the first two revealed the presence of dihydrochalcones as the predominant components. Dihydrochalcones are abundant in the apple genus *Malus* (Williams 1966). Phloretin was the major constituent of the methanol solubles, while the ethyl acetate solubles contained phloretin, phloridzin, acetyl phloridzin (fig. 6.6-2), and other related compounds

Phloretin, R = H
Phloridzin, R = glucosyl
Acetylphloridzin, R = acetylglucosyl
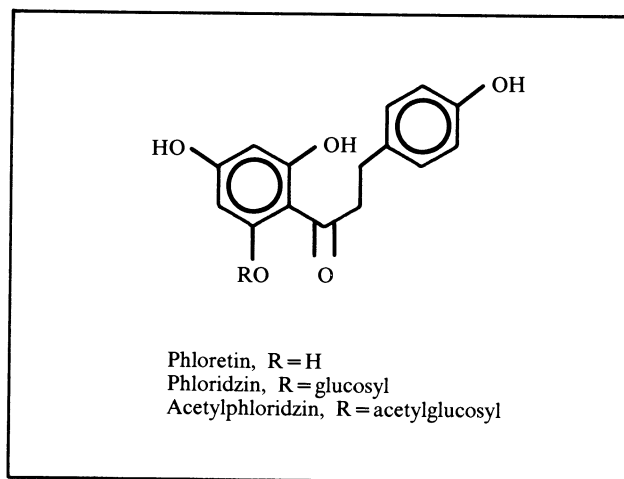
Figure 6.6-2.—*Dihydrochalcones isolated from* Kalmia latifolia.

(Doskotch et al. 1977d). None of these, however, was significantly active, in spite of the fact that these compounds have toxic properties. For example, phloridzin induces a strong glucosuria in mammals and has been used in physiological studies (Crane 1968). It is also an inhibitor of glucose transport across certain cell membranes (for example, erythrocytes) and serves as a feeding deterrent for two nonapple feeding aphids (Montgomery and Arn 1974), yet is a probing stimulant for apple-feeding aphids.

As the major constituents were removed from the active fractions, it became apparent that the activity was associated with the minor nonphenolic components. At present, a total of four potent compounds has been isolated in crystalline form. Their characterization is incomplete, but they are members of a class of diterpenes known as grayanotoxins (Gasa et al. 1976) that occur in the Ericaceae. Feeding was reduced below 50 percent when these substances were tested at doses of 50 μg per milliliter or less.

Melaleuca leucadendron L. (*Myrtaceae*)

The most active partition fractions of the leaf extract of *M. leucadendron* were the hexane and methanol solubles. Separation of the former material on a silicic acid column gave a heavy oil, as the most potent component, which was characterized as the sesquiterpene alcohol, nerolidol (fig. 6.6–3) (Doskotch and Cheng 1977). Although the compound is not highly active (50 percent feeding at 4 mg per milliliter), it is present in relatively high concentration and accounts for the deterrency of this source.
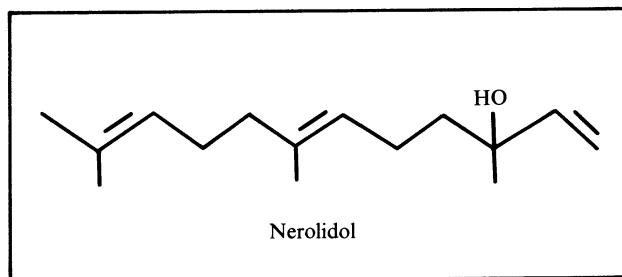
Figure 6.6–3.—*Feeding deterent from* Melaleuca leucadendron.

Catalpa speciosa Warder (*Bignoniaceae*)

The ethyl acetate and *n*-butanol solubles were the antifeeding fractions after partitioning of the crude extract of *C. speciosa* foliage. Both were subjected to column chromatography. The ethyl acetate solubles, with an activity of 23 percent at 6 mg per milliliter gave eight column fractions, none of which were comparably active, yet recovery of sample was complete and no alteration of constituents was detected as monitored by thin layer chromatography. A similar result was obtained with the *n*-butanol solubles, but in this case testing of the recombined column fractions was performed. The results (table 6.6–3) clearly show that the activity of the *n*-butanol soluble fraction is due to the combination of a number of weakly active or inactive components (Doskotch and El-Naggar 1977). *Catalpa speciosa,* therefore, does not appear to be a good source for individually active deterrents.

Summary

A sound beginning has been made in the area of natural products that affect the feeding behavior of the gypsy moth larvae. A reliable bioassay has been developed that is based on a cellulose-agar basal diet to which is added a plant extract and offered to third-instar larvae. Test results reflect observations in the field. The feeding behavior is controlled by substances that can be designated as stimulant, deterrent, or synergist. Fractionation of specific plants with deterrent activity has produced a number of pure constituents. To date, these have been either known substances or new members of known classes.

Like any screening program, the ultimate success of finding an effective control agent rests on the total number of leads that can be followed. Invariably some will fall by the wayside early in the isolation, either because of loss of original activity, spread of activity over an inordinate number of fractions, or inability to consistently reproduce activity. In those cases where pure constituents are isolated and characterized, other problems will have to be overcome. An adequate supply may not be available via isolation, or chemical

Table 6.6–3.—*Feeding response to* n-*butanol soluble column fractions of* Catalpa speciosa

Column fraction[1]	Weight (mg)	Dose[2] (mg/ml)	Feeding percent[3]	Recombined fractions[4]	Dose[2] (mg/ml)	Feeding percent[3]
A	380	0.45	73	A, B, C	2.1	38
B	280	.33	79	D, E, F	5.0	53
C	950	1.08	92	G, H	0.5	66
D	1900	2.03	77			
E	650	1.50	85	D, E, F, G, H	5.5	46
F	320	.75	65	A, B, C, G, H	2.6	52
G	170	.20	89			
H	210	.25	85	A to H	7.6	43

[1]Silicic acid column fraction from 5.5 gm of *n*-butanol solubles exhibiting activity of 40 and 24 percent at 6 and 10 mg per milliliter, respectively.

[2]Concentration of residue incorporated into the cellulose-agar red oak diet.

[3]Feeding on control diet of red oak was taken as 100 percent.

[4]Recombination was in approximate relative proportion of fraction in crude.

synthesis may not be economically feasible. However, structure-activity investigations may give clues to the active features of a compound that could suggest simple structural analogues.

In the end, it should be possible to introduce (after approval by regulatory agencies, of course) specific potent inhibitors with minimal disturbance of the ecological balance. All of this will, of course, take time and concentrated effort. In addition to the obvious value of feeding deterrents, there is need to know about the nature of the stimulants and synergists. No research has been done on feeding stimulants, and little information is available on these. Stimulants could find use as components of already effective pesticide preparations, which are active on ingestion; this would insure adequate uptake or would allow a lowering of the amount of pesticide applied. Recognition of feeding stimulants could lead to synthesis of effective antagonists, and in addition, plant breeders would be able to screen agricultural, horticultural, and forest crop varieties for the presence of stimulants and to direct their efforts toward selection of stimulant-lacking strains. In the long run, this would be most effective way to combat the gypsy moth. There are many avenues to pest management, and chemical knowledge about feeding should provide several. As a byproduct, chemical, photochemical, and biological information of purely academic interest would also accrue. Campbell (1975)

put it well in his booklet on the gypsy moth: "We need to know the enemy better."

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6.7 Sterile–Male Technique

Victor C. Mastro, Charles P. Schwalbe, and Thomas M. ODell

Introduction

Interest was focused on the sterile-male technique (SMT) for insect population manipulation with the successful eradication of the screwworm, *Cochliomyia hominivorax* (cqrl.) on the island of Caracao (Baumhover et al. 1955). Since that time, application of the technique (or modifications of it) has been attempted with a large variety of insects. The degree of success of these applications has been wide in scope, and excellent recent reviews of the subject are available (Whitten and Foster 1975, North 1975, Proverbs 1969).

Knipling (1955), who is generally credited with propounding the SMT, outlined several criteria an insect should meet before the technique could, in his view, be successfully applied. The gypsy moth, *L. dispar,* appeared to meet some of the criteria and research was initiated shortly after the screwworm success was reported. The earliest work focused on defining the proper dose and timing of irradiation treatment that would induce sterility. Field trials followed to test the usefulness of the technique and the competitiveness of male moths. These early trials were severely hampered by the lack of artificial diets and rearing techniques necessary to produce quality insects for testing. Shortly thereafter, work was also initiated on chemical sterilization of males and complementary field trials to test for competitiveness. During this period, researchers were optimistic about success (Knipling 1969).

Additionally, some European studies were carried out with radiologically sterilized males. In the United States, work on developing the SMT for the gypsy moth essentially stopped in the early 1970's for various reasons. These included the lack of a rearing facility and technology for producing "quality" insects, inadequate assessment techniques, and the inconclusive and often discouraging results of early field trials.

Interest was refocused on the possibilities of using SMT for gypsy moth population suppression under the guidance of the Expanded Gypsy Moth Program. Because of program accomplishments, which included development of mass-rearing technology, the feasibility of using SMT on the gypsy moth was revaluated. LaChance (1976) reviewed past work on the gypsy moth and outlined problem areas, and also made recommendations for reassessing the feasibility for application of the SMT. Three major areas of importance in developing SMT technology were identified: The capability of mass rearing quality insects, the development of techniques to assess male competitiveness, and the capacity to evaluate the impact of a sterile male release on a pest population. Early in 1977, with impetus from the report by LaChance, the Expanded Gypsy Moth Program formally initiated a reinvestigation of the SMT.

This section outlines what has been accomplished and points out areas where difficulties may arise. Although it is too early for a report on the findings of the current project, objectives and strategy are outlined, and speculations on some preliminary results are also presented.

Radiological Sterilization

Investigators began to explore the effects of gamma radiation on gypsy moths as early as 1957 (Godwin et al. 1964). These studies, carried out over several years (1957–61), were designed to determine the proper radiation dose and insect development stage that would produce sterility with as little somatic damage to the insect as possible. Various age classes of pupae and late-instar larvae were irradiated with doses ranging from 0.5 to 20 krads (cobalt-60). Degree of sterilization was determined by mating treated insects with untreated insects and checking egg embryonation and hatch. Radiation-induced damage to the insects was measured by larval and/or pupal mortality and morphological abnormalities in adults. Length of pupal stage and adult longevity were also considered. Results of the first year's studies indicated that irradiating male pupae 7 days old or older with 20 krads caused sterility with little pupal mortality, and no adult morphological abnormalities. Exposing younger pupae to the same dose

Table 6.7-1.—*Percentage of eggs hatched[1] when male gypsy moths were subjected to several radiation treatments and mated to nonirradiated females[2]*

Dose (krads)	Treatment, by pupal age (days) when irradiated							
	(Godwin et al. 1964)		(McDonald 1966-67)		(Vasiljević 1967, 1970)		(Maksimović 1971, 1972a)	
	7+	9-11	9	9	7-13	7-13	8-13	7-13
1					95.0	83.0		
2.5	72.7	7.4						
4					79.0	77.0		
5	38.8	2.4						
7					79.0	77.0		
10	48.6	.4						
15			16.5		44.0	64.0		20.1
17.5			5.4					
20	2.4	0	2.9	0.36	29.0	29.0		10.2
22.5			2.1	.10				
25			.5	.06	10.0	2.4		
27				0				
27.5				0				
30					.77	3.3	0	
40						0		
50						0		
60						0		
Control	51.7	6.8	38.4	32.9	80	73	82.6	48.4

[1]Computation of this percentage varies from percent of total eggs hatched to percent of embryonated eggs hatched.

[2]Rearing conditions and diet of larvae differed greatly (see text).

dramatically increased pupal mortality and adult morphological abnormalities. A dose of less than 20 krads did not induce high levels of sterility. Results of subsequent experiments confirmed these results, and it was concluded that irradiating male pupae (more than 9 days old) with 20 krads produced a high level of sterility without evident somatic damage.

Rule et al. (1965) explored the timing and sequence of spermatogenesis in the gypsy moth and the effects of irradiating fourth- and fifth-instar larvae with 0.5–20 krads. His results showed that, although various stages of spermatids were present in fourth-instar larvae, fully formed spermatozoa were not present until the fifth instar. When irradiating these late-instar larvae, he found that, in addition to somatic damage expressed as morphological defects in the adult stage, there were changes in the epithelium of the testes, the spermatozoa, and the germinal cysts. Depending on the stage exposed, low doses of radiation (0.5 and 1 krad) produced shattering of the

sperm bundles and abnormally shaped sperm. Higher doses (10 and 20 krads) produced degenerated germinal cysts and spermatozoa. In addition, a plugging of the entrance funnel of the vas deferens was noted. These results are in general agreement with those results of similar studies on other insects. Generally, more somatic damage can be expected when early stages are irradiated, and radiation is more injurious to early stages of spermatogenesis (Proverbs 1969).

Studies by Godwin and Rule, as just summarized, were conducted on insects that had been collected in the field as eggs (in 1957 and 1961) or larvae (1959) and reared on oak foliage in the laboratory. Insects reared in this manner were reported to be smaller than field-collected pupae. To further confound interpretation of the results, the eggs produced by mating untreated insects had a low incidence of hatch in the second year of the study (1959). It is difficult to determine what other effects this rearing procedure

may have had on the quality of the insects studied, but apparently something in the handling procedures and/or nutrition of these insects was not optimal. It was not until 1965 that an artificial diet was available for rearing gypsy moths (ODell and Rollinson 1967).

McDonald (1967) conducted the first irradiation studies on insects to be reared on artificial diet. His purpose was to further define the optimal radiation dose required to produce sterile males. Pupae were irradiated when 9 days old with 15–27 krads. These studies are incomplete, but his preliminary results indicate that a 15-krad treatment reduced egg hatch to about 15 percent. A 20-krad treatment further reduced hatch to approximately 3 percent. However, a control group had only 38 percent hatch. Treatment with 20 krads did not noticeably increase the incidence of pupal mortality or adult abnormalities; however, treatment with higher doses of 27 and 27.5 krads did.

Vasiljević (1970) also investigated the effects of irradiation on male and female gypsy moths. He chose to work with a wider range of treatments and exposed pupae to 1–60 krads; unfortunately, the age of the insects treated is not discussed. Results of mating irradiated females with untreated males indicated that female sterilization could be achieved with doses of less than 7 krads. However, mating of irradiated males with untreated females indicated that males were more "radio-resistant" and that higher doses were required for sterilization. Vasiljević's results differed from those of previous research, in that he found untreated females mated with males exposed to 25 krads and 30 krads produced eggs that hatched. Unfortunately, the fate of the hatching progeny was not discussed. He concluded that exposure to 30 krads is the lowest safe limit to produce complete male sterility.

Maksimović (1972a) recognized that the conclusions of Godwin's, McDonald's and Vasiljević's studies (table 6.7-1) were inconsistent on how much radiation was required to produce sterility without pupal mortality and adult abnormalities. In a field evaluation of males treated with 30 krads, Maksimović (1970) found them not competitive. In subsequent tests (Maksimović 1971), male pupae in three age classes (2–5, 6–10, and 11–15 days old) were irradiated with either 30 or 40 krads from a CO^{60} source. Adult longevity, sterility, duration of copulation, and effects on untreated females mated to treated males were studied. Results appear to be in closer agreement with those of Godwin et al. (1964) and McDonald (1967). Higher radiation doses produced a high incidence of mortality in the pupal stage, and somatic damage was expressed as a large percentage of adult abnormalities; the younger the age class of pupae irradiated, the more pronounced the effects. He also noted negative effects of radiation on the length of male adult life and the duration of copulation. As part of this study, Maksimović explored the relationship between size of male moth (body length) and the ability to mate. Generally, he found larger males mated more frequently. He concluded that radiation doses of 30 to 40 krads had a harmful effect on males and recommended that lower dosages for either complete or partial sterilization should be studied. Maksimović (1972a) pursued this tack in a later study and irradiated male pupae with 15 and 20 krads. These males, in contrast to those used in an earlier study, were reared on an artificial diet. He found, generally, that untreated females mated with treated males produced few eggs that hatched: 20 and 10 percent hatch for 15-krad and 20-krad treatments, respectively.

Irradiated males were capable of several matings and elicited normal egg-laying behavior of untreated female mates. In addition, irradiation treatment did not shorten a male's life span. Size was implicated in male competitiveness; larger males, irradiated or nonirradiated, lived longer. He further speculated that lower doses of radiation might produce an even more competitive male that would sire sterile offspring.

Field Trials With Radiologically Sterilized Males

Results of SMT field trials using radiologically sterilized male gypsy moths have met with varying amounts of success. In theory, SMT is best suited for application against low-density populations. It is extremely difficult to quantify these sparse populations and measure net changes resulting from sterile

male releases. Additional complications arise when male migration is considered. A major problem encountered in early field trials was that of questionable male quality. The earliest studies used field-collected insects for test purposes, and although efforts were made to collect "quality males," results of releases varied dramatically. This problem of variable and questionable insect quality continued to affect field trials even after laboratory cultures were established.

Merriam (1963) conducted the earliest field trials using radiologically sterilized males in artificially infested plots. Field-collected males, irradiated as 9-day-old pupae with 20 krads, were released to give overflooding sterile-to-fertile ratios of 10:1, 17:1, and 38:1, plus a control. Sterilized insects were released as adults while untreated males and females were placed in the plots as pupae.

Results of these tests were not conclusive. The proportion of egg masses that had some hatch was nearly the same for all treatments; however, the proportion of egg masses with less than 10-percent hatch was about 69 percent, 72 percent, and 52 percent for the 38:1, 17:1, and 10:1 sterile-to-fertile insect ratios, respectively. Merriam concluded that treatment with 20 krads may not have completely sterilized males, which resulted in egg masses with partial hatch.

Collier and Downey (1965a) initiated a project to study how released males would interact with a field population. To study this interaction, marked males, emerging from field-collected pupae in the laboratory, were released from the corners of a square plot. To monitor male activity, 100 traps baited with feral female pupae were placed in the center of the plot. In addition, 100 untreated male pupae were placed in the central 0.4-ha area. Of the approximately 1,600 marked males released, 17 were recovered, while only two unmarked males were trapped. It was theorized that if the marked males had been sterile, a dramatic reduction would have occurred. Perhaps the most important observation in this study was that males were active in the test area for only 2 or 3 days after release. This was one of the first indications of what a male moth's lifespan would be under field conditions.

Collier (1967) pursued this comparison. Using a small uninfested island as a test area, he compared the competitiveness of four groups of male moths: Untreated males reared on an artificial diet, untreated males field collected as pupae, field-collected males exposed to 27.5 krads as 9-day-old pupae, and field-collected males sterilized with tepa (discussed in a later section). Three releases were made of equal numbers of the four groups of males. Activity was monitored with traps baited with virgin females.

Collier found that males reared on artificial diet were captured in traps in numbers greater than the three other groups tested. Fifty of the untreated laboratory-reared males were recovered, compared to 35, 35, and 23, respectively, for the untreated field-collected males, those treated with tepa, or those treated with 27.5 krads. It appeared that the radiologically sterilized males were less competitive. Collier stated that 22 percent of the males eclosing from pupae in this group had malformed wings, indicating somatic damage from radiation treatment. Results of McDonald's 1966 test, which indicated that lower doses (22.5 krads) were completely sterilizing without evident somatic damage, unfortunately were not available to Collier until this test had been completed.

Collier (1968) in 1967 continued to pursue sterile male work and moved his testing site to large tracts of continuously forested areas. All males used in this series of tests were reared on an artificial diet. Males were sterilized as 9-day-old pupae with 25 krads from a CO^{60} source. This was a decrease in radiation dose from the previous year's treatment; Collier stated, "less wing damage and greater competitiveness... was anticipated." Areas used for these trials were naturally infested. A 40:1 overflooding sterile-to-feral male ratio was chosen. Native male-moth density was estimated by extrapolating from the previous season trap catches. Nine separate sites were chosen as test areas and treated with a pesticide when gypsy moth larvae were in the second and third instars. All plots were to be monitored with traps and by sampling egg masses for hatch.

Results of these tests were inconclusive. In several plots, no egg masses could be found, and only low

numbers of released moths were recovered. In one plot, although no released males were recovered in traps, 11 egg masses were collected, several of which had a very low percentage of hatch.

A series of field trials (Statler and McLane 1968, Godwin 1972, Morris 1972) were conducted throughout this period. Although different experimental designs, sources of insects, and evaluation techniques were used, the trials all had the same purpose: To evaluate the impact of a sterile-male release on a sparse natural population. Results of all of these trials were inconclusive. Poor-quality insects for test purposes complicated analysis of the results. Two factors, however, made analysis of these tests impossible; reliable techniques for estimating gypsy moth density in a sparse population and for quantifying the effects of a sterile male release were not available.

Statler and Downey (1969) continued an evaluation of laboratory-reared insects and the effects of irradiation on male competitiveness. In 1968, three groups of males were chosen for comparison. Two groups of moths had been reared in the laboratory on artificial diet; one of the groups was exposed to 20 krads of gamma radiation as 9-day-old pupae, while the other was left untreated. The third group was collected as pupae from the field and held in the laboratory until eclosion.

To compare the competitiveness of these three groups of males, they were released from the center of plots, and laboratory-reared, virgin female baited traps were checked at about 12, 36, and 52 hours after the release. Males were released in plots in paired groups (that is, irradiated laboratory-reared vs. feral untreated; irradiated laboratory-reared vs. untreated laboratory-reared). In all, six replicates were completed for each of the paired releases. Three hundred males of each group were released in each replicate.

The authors concluded from these trials that laboratory males treated with 20 krads were less competitive than untreated laboratory males and that the irradiated laboratory males were also less competitive than feral males. These results were verified in tests in 1969 in which large-size plots (about

4 ha) and a slightly different trap arrangement were used. Results were subjected to statistical analysis, and the aforementioned differences were found to be significant at the 1 percent level of confidence.

Maksimovic also carried out a series of field studies with sterile males (Maksimovic 1970, 1972b, 1974). Sterilized males for these studies were released in a park on an island for 3 successive years. In the first year of the study (1969), male pupae were irradiated with 30 krads from a CO^{60} source. In the next 2 years, doses were lowered to 20 krads for treatment of male pupae 9-13 days old. The ratios for sterile-to-feral males were estimated to be 1:2.0, 1:2.4, and 1.55:1 for the 3 successive years. Feral male densities were estimated by counting all of the egg masses in the test area. It was assumed that 95 percent of the egg masses were found and that a 1:1 sex ratio existed. To monitor the effects of releases, egg-mass densities were estimated yearly. Fecundity and hatch data were also collected. Data from the release area were compared to similar data from an area where no treatment had been applied.

Maksimović noted that populations in the treated area declined, while those in the control area increased. He credits this as "a result of the cumulative effect of the releases of irradiated males." However, populations around the treated area also decreased in 1971. Egg masses collected from the area of sterile male release had a lower hatch rate than egg masses from the control area. However, egg hatching in the control area was found to be "different and changeable," and Maksimović concluded this resulted from ecological conditions and food supplies. The evidence that the observed population decline resulted from these releases is not conclusive.

Induced Inherited Sterility

The advantages of releasing partially sterile insects in a suppression program have been extolled by many. The benefits generally cited are that insects treated with substerilizing doses suffer less somatic tissue damage than fully sterilized insects and are thus more competitive; the F_1 progeny from partially sterile × wild crosses would be reared in the field and

therefore would be more hardy and in synchrony with the native population; and there would be a bonus effect of continued population suppression after the initial release. This last topic has recently been reviewed by North (1975), who pointed out that in Lepidoptera the F_1 progeny of irradiated parents tend to develop more slowly than progeny from untreated individuals and also that male progeny of irradiated males often fail to transfer sperm to the spermathecae properly.

Past studies of induced partial sterility with the gypsy moth are limited. Statler (1969) and Paszek (1968) both reported some preliminary results on studies with F_1 sterility. Statler treated pupae with 0.5 krad, 2 krads, and 8 krads of gamma irradiation and found sterility in the F_1 and F_2 progeny. Paszek irradiated 9-day-old male pupae with 20 krads and found sterility when the progeny were intermated for two generations. He also reported a distorted sex ratio (2:1) in favor of males for the F_2 generation. Hatching rates of egg masses for a control group were low, making comparisons difficult. Clearly this area of knowledge will require further investigations to determine the benefits of using partially sterile insects in an operational program.

Hybrid Sterility

A theoretical discussion of the possibilities of using cytoplasmic incompatibility for suppression of the gypsy moth in the United States was presented by Downes (1959). Building on the classical studies of sex determination reported by Goldschmidt (1934), Downes explored the possible use of strong-race males for population suppression. (The use of strong race or weak race refers to the potency of the sex determining factors.) Within a race, the sex determining factors are in harmony, and mating produces normal fertile offspring. However, when interracial crosses are made, female-male intersexes result. Downes suggests that the strain of gypsy moths in the United States originated from a half-weak race in France. If females of this race are mated to males of a strong race (Japanese), the male progeny should be normal and fertile, but the female progeny would be

middle-grade intersexes and sterile. Simply, the proposal then is to rear and release strong-race males. A bonus of this technique is that F_1 male progeny, when mated with native females, would continue to produce intersexual sterile females. Downes also points out some difficulties that may be encountered when considering this approach. Strong-race males may not be competitive with native males, and all the behavioral and physiological components influencing competitiveness would have to be considered. Also, genetic material from the strong race introduced in the American race may make it more successful in its environment. Downes suggested this risk could be guarded against to some extent by testing progeny of experimental crosses before liberation.

Leonard (1978) demonstrated that, when strong-race males were crossed with weak-race females, the progeny were female sterile intersexes and fertile viable males. However, when F_1 male progeny were mated to weak-race females, only about 50 percent of the female progeny were sterile intersexes; the remaining 50 percent were fertile. Leonard advised against the release of strong-race males into the United States because the addition of Japanese genomes to the population could possibly extend the ecological limits of the native strain.

Chemosterilization

Testing chemosterilants for the gypsy moth began in 1963. Collier and Downey (1964, 1965b, 1967) screened several compounds as sterilants. These compounds were applied to various life stages of field-collected insects with different application procedures. The results of these studies indicated tepa (tris (l-aziridinyl) phosphine oxide) was best suited as a sterilant when adult males were exposed to a residual film. This treatment resulted in the greatest degree of sterility without obvious deleterious effects or excessive mortality. Pupal dips produced either excessive mortality when applied to pupae less than 24 hours old or no sterility when applied to pupae greater than 24 hours old. When moths were exposed to residual films of metepa (tris (1-(2-methyl aziridinyl)-phosphine oxide)) sterility was achieved but mortality

rates were greater than those for tepa. Several other compounds were screened for activity but were rejected because of low activity or deleterious effects. It was concluded that the most effective treatment was exposure of males to a residual film of 1 mg tepa per 100 cm^2 for 8 hours. Other results indicated that increasing the time between treatment and mating or lengthening treatment increased the proportion of eggs with no hatch.

Several field studies were conducted to evaluate male moths that had been treated with tepa. The earliest studies, described by Collier and Downey (1965*b*), were carried out in small uninfested plots in 1964 and 1965. Males used in these studies were field collected as pupae and allowed to emerge in the laboratory. Moths were exposed to a residual film of 8 mg tepa in a 3.7-1 bottle for 8 hours. One hundred traps containing live virgin females were placed in concentric rectangles within 0.4-ha test plots. In 1964 and 1965, two replicated releases of tepa-treated and untreated males were made. In 1964, a mean of 24 percent and 32 percent of the tepa-treated moths and untreated moths were recovered, respectively. In 1965, 27 percent of the tepa-treated males and 17 percent of of the untreated males were recovered. A check of egg masses laid by females in traps in 1964 where a tepa-treated male was caught resulted in egg hatch of 6 percent. The conclusion was that tepa-treated males were able to search out and locate females as well as untreated males.

Collier (1966) continued an evaluation of tepa-treated males on three uninfested islands in Lake Champlain. On the largest island (32 ha), five releases of tepa-treated and untreated males were made between July 11–19. A total of 3,768 tepa-sterilized and 112 untreated males were used for a 34:1 sterileto-feral ratio. Observers checked females placed in either open containers or traps for mating pairs. Males were collected for determination of male treatment. In all, 60 tepa-sterilized males and no untreated males were observed.

After this last series of field trials, work with chemical sterilants because of environmental and handling considerations was terminated, and the emphasis shifted to radiologically sterilized males.

Current Status of the Gypsy Moth Sterile-Male Program

Clearly, past work on SMT for the gypsy moth was undertaken when conditions were not favorable for success. The biology and behavior of the insect were not clearly described, mass-rearing techniques had not been developed, and techniques for comparing male competitiveness were limited. However, the work was initiated at a time when the screwworm program, lacking much of the same knowledge, was a resounding success. Only recently have scientists involved with sterile-male programs realized some of this information is critical for long-term success (Bush and Neck 1976). Within the constraints imposed by the voids in knowledge and technology described above, the early research and field trials did provide a foundation for future work. With the additional contributions of the current gypsy moth program, including a mass-rearing capability and a fuller understanding of gypsy moth behavior and biology, a revaluation of the SMT was directed by Expanded Gypsy Moth Program staff.

In his evaluation, LaChance (1976) made several recommendations for the initiation of a sterile-male gypsy moth development program. The first recommendation was that "a *long-range* research program designed to *assess the feasibility* of using sterilized male gypsy moths as a method of suppressing and eliminating newly established or isolated low-density populations of gypsy moths be immediately designed and implemented" (emphasis added). An effective sterile-male research program must begin with a concentrated effort to describe the behavior of feral populations (Leppla and Chambers 1977) and to develop techniques for comparing competitiveness of colonized and feral insects. An equally important objective for success of a sterile-male program is to develop techniques for evaluation of the impact of field releases of sterilized males. Whitten and Foster (1975) elegantly stated the need for this type of approach: "Ecologists now associated with Sterile Insect Release Method (SIRM) need little convincing of the importance of thorough, long-term ecological research before eradication or suppression

attempts are initiated. However, by continuing to propose short-term evaluation studies, some entomologists have created an atmosphere in which many have come to expect practical results with SIRM during time intervals in which other methods of control rarely make significant progress."

With the primary objective of assessing the feasibility of using the SMT for suppression or elimination of the gypsy moth in mind, the following research areas were outlined. The first is to characterize the field behavior of feral gypsy moths. Several workers have reported on aspects of gypsy moth behavior, and Doane (1976) reviewed this area of research. Although much has been learned, several behavioral parameters exist that still require qualitative and quantitative characterization. These include dispersal, temporal and spatial activity periodicity, and mate finding in various population densities. In addition, little information is available on how environmental parameters affect field behavior.

To complete characterization of feral populations and to eventually evaluate the effects of a sterile male release, techniques for monitoring adult moth activity must be developed. In the past, traps baited with synthetic pheromone and live female gypsy moths have been used as survey tools and as devices to assess mating disruption tests (Granett 1976). Controversy, however, has arisen over the reliability of these techniques and the interpretation of trapping data. The use of laboratory-reared females as a measure of mating disruption has been questioned because of their pheromone-release characteristics (Richerson and Cameron 1974) and their decreased attraction in the field (Richerson 1976).

In addition, placement of females in traps of some designs reduced a female's attractancy. Racemic disparlure-baited traps are less attractive than a calling feral virgin female (Cardé et al. 1974, Mastro et al. 1977), and thus their use is limited. However, recent evaluation of the (+) enantiomer of disparlure has demonstrated that it is a more powerful attractant than the racemic mixture (Plimmer et al. 1977, Cardé et al. 1977), and it may be used as a monitoring tool. Results of a 1977 SMT field study indicate that traps baited with (+) disparlure are equivalent to a feral

virgin female in attractancy in terms of numbers of males captured. However, it is essential to assess, qualitatively and quantitatively, the response of males to the simulated call, relative to the natural female call, before accepting the trap as a tool for monitoring male behavior. Ultimately, demonstration of the SMT will need to be related to changes within a target population. The only technique currently available is checking egg masses for hatch.

The recent positive developments in rearing technology for the gypsy moth have improved the quality of insects, and this was one of the main stimuli for initiating the present SMT research program. Because previous procedures for producing sterilized male gypsy moth were somewhat inconsistent, and insects treated had been reared under different environmental and nutritional regimes, this rearing technology has provided the means for pursuing another objective of the current research program: Defining the optimal radiation dose and appropriate insect life stage to produce competitive sterile males. Included in these studies is a fuller investigation of induced partial sterility. Although the results of these studies are still preliminary, it appears that sterility can be achieved without obvious morphological abnormalities and adverse effects on male competitiveness.

The ability (quality) of the laboratory-reared male gypsy moth to compete with its feral counterparts in the field has received only cursory attention. Richerson and Cameron (1974) found that laboratory-reared gypsy moth males were not as sexually aggressive as feral males and questioned their use in trapping and mating disruption studies. Developing performance standards and methods for monitoring male quality in the laboratory is the third major research aspect of the current program. Specific male competitiveness traits will likely determine how well a male strain performs in the field. Inadvertent selection of deleterious behavioral characteristics can impair the ability of the reared male to compete with the wild counterpart. The advantages of monitoring these traits in the laboratory are that individual components affecting male competitiveness can be manipulated and assessed; specific areas of deficiency can be

identified; year-long evaluations of insect quality can be made before limited resources are committed to a field release; and a system of continuous feedback to the rearing facility can forewarn of problems and allow corrective action to be taken. Based on preliminary data from 1977 field trials, laboratory studies will be conducted to assess the behavioral parameters that may affect the competitiveness of male gypsy moths. These parameters are response of male to behavioral stimuli (olfactory and visual), ability and propensity for flight, the ability to mate and transfer sperm, and the periodicity of flight and sexual activity. At present, other components of quality such as fertility, fecundity, size, and developmental rate are being routinely monitored and are the subject of discussion in other sections of this volume.

In 1977, in cooperation with scientists at the ARS Basic Biology and Attractants Laboratory, Gainesville, Fla., several laboratory techniques were assessed for usefulness in evaluating the quality of male gypsy moths. Laboratory-reared male moths were tested utilizing the following techniques and equipment: An actograph for assessing periodicity of activity and vigor of lab colony (Leppla and Spangler 1971); a flight mill for measuring duration, velocity, distance, and frequency of flight (Sharp 1976, 1977); and the electroretinogram method for detecting visual differences (Agee 1977). In addition, Baker and Cardé (1978) have utilized a flight tunnel for investigating the in-flight behavior of male gypsy moths. Each of these techniques provides specific measures of insect activity. Evaluating their usefulness for assessing male performance relative to field competitiveness is a major objective of the 1978 SMT program. The constraints of simplicity and economy listed by Huettel (1976) will have to be considered in the evaluation; some will be essential, others may require modification.

Summary

It would be impossible to predict what the outcome of the current evaluation of the SMT for suppression or elimination of the gypsy moth will be; only 9 months have elapsed since the initiation of this project. However, the following current developments tend toward optimism.

Laboratory-reared male insects are now of a higher quality than in the past. In 1977 field trials, laboratory-reared males were able to locate pheromone sources as well as their wild counterparts. Longevity of laboratory males also appears to be nearly the same as wild strains. Development of rearing procedures for producing high-quality insects economically is continuing. Moreover, the rearing technique and knowledge of the basic biology obtained thus far will provide the tools for studying the effects of radiation on gypsy moths. Research can now be carried out on induced partial sperility, sperm transfer, and egg fertilization without the difficulties and drawbacks of collecting field material or losing the results of a test because the requirements for diapause were poorly understood.

Perhaps the major reason for optimism for success of the SMT is the cooperative spirit that prevails among the three Federal agencies involved—the Science and Education Administration, the Forest Service, and the Animal and Plant Health Inspection Service. This commitment to a long-term program of careful evaluation of the feasibility of the development of the necessary knowledge and techniques for implementation of the SMT has been primarily the result of direction from gypsy moth program staff.

The goals that the current program views as prerequisites to operational use of the SMT for the gypsy moth are: The ability to rear large numbers of "quality" insects economically, including development of the technology for sexing, marking, and harvesting; definition of the basic components of male behavior that contribute to competitiveness; development and incorporation of laboratory quality monitoring techniques that can be related to field performance; development of methodology for measuring and analyzing the results of field releases of sterilized males; and the development of shipping, handling, and delivery systems compatible with the scope of anticipated programs. The development of this information should provide a realistic evaluation of the SMT for the gypsy moth before operational use is considered.

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Socioeconomic Impacts

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William B. White and Noel F. Schneeberger

Introduction

Forest insect pests such as the gypsy moth affect the productivity, uses, and esthetic values of forest resources in many ways. The long-term cumulative net effects, or impact, limit and disrupt all phases of resource planning and program execution.

For decades, definitive data on the effects of gypsy moth invasion and the varied consequences of defoliation were lacking. Prior to and during the Expanded Gypsy Moth Program, studies were directed at thoroughly evaluating the complex socioeconomic impacts created by this pest. This information is critical to the development of benefit/cost analyses, which in turn are essential to those who make pest-management decisions.

Definition of Impact

Insect-caused impact can be divided between ecological and socioeconomic components, which are, unfortunately, often in conflict with each other. The ecological component can be defined as "the cumulative net effects of insects on the ecological parameters of forest stands, or other land management units containing trees" (U.S. Department of Agriculture, Forest Service 1972). These cumulative net effects that insects have on individual trees result, over time, in quantitative and qualitative changes in stand parameters such as stocking density, net volume, annual growth rate, species composition, and appearance. The measurement and analysis of these effects constitute the basic data of the *ecological* component. The *socioeconomic* component of impact considers how the effects influence management objectives and forest resource values, including recreational and esthetic, which are important to the public.

The terms *injury* and *damage*, often used in place of impact, are supportive to the overall concept of insect-caused impact. *Injury* is used to describe something that is physically or physiologically wrong with the tree; *damage* interprets that injury through some value system. For example, a tree that dies after defoliation by gypsy moth larvae reflects injury. The loss in cubic meters per hectare of pulpwood reflects damage according to the human value system. The socioeconomic impact of this damage is dollars per hectare, whereas the ecological impact might be expressed in terms of modified species composition or reduced stocking level. Impact, therefore, is not damage but the *significance* of damage.

Impact is a dynamic variable that is a function of insect-induced changes in forest stand conditions and on the criteria established for particular management objectives. Its definition can vary according to geographic region, ecological and economic situations, current forest management practices, resource uses and potentials, and people involvement (U.S. Department of Agriculture, Forest Service 1972). In 1972, a workshop was held in Marana Air Park, Ariz. The purpose of the meeting was to discuss the impacts that insects and diseases have on uses, values, and productivity of forest resources. The participants, from all regions of the country, were divided into groups, each of which gave their own definition of impact.

The Douglas-fir work group developed a definition that was typical of most of the other groups: "Pest impact is the net effect by pests on any forest resource that requires a management action change now, or in the future." This implies that management objectives are previously set for forest resources (especially timber), and this is the standard against which impact is measured. The eastern hardwoods work group, on the other hand, described impact in terms of people involvement, a function of public pressure: "Impact occurs when effects of insects and diseases result in a response by people." The concept of impact formulated by the remaining groups fell between the two described.

As a result of the Marana workshop, impact was redefined as "the cumulative net effect of insects and diseases, that results in modification of management activities for specialized forest resource uses and values" (U.S. Department of Agriculture, Forest Service 1972). However, because the individual work

681

groups pointed out that the definition of impact is dependent upon many diverse factors, it was agreed that the explicit definition of impact and the way in which it is to be measured must be specified in each particular situation. Also, the criteria used in each case must apply to a particular time or planning period.

The unique definition of impact as stated by the eastern hardwood work group is very applicable to the current gypsy moth infestation in the Northeast. Within the known boundaries of the gypsy moth infestation are some of the most densely populated areas in the East and are, therefore, areas where extensive people/forest interface occur. In these situations people regard trees as having intrinsic real estate, recreational, and esthetic values that commonly exceed the trees' market value for timber.

Impact Assessment

It has been only in recent years that the socio-economic impacts of the effects of various insect pests have become an important issue. This can probably be attributed in part to the publicity concerning the gypsy moth, southern pine beetle, and Douglas-fir tussock moth programs (the Combined Forest Pest Research and Development Program), and the subsequent need to quantify both the costs and benefits of controlling these pests. In 1974, a proposal for the emergency use of DDT against the tussock moth resulted in an exhaustive Environmental Statement investigating the beneficial and detrimental effects of using DDT, and a subsequent benefit/cost analysis assessing the possible control alternatives (U.S. Department of Agriculture, Forest Service 1974).

Such impact assessment has by no means been limited to these insect problems. In 1976, for example, South Dakota, Colorado, Wyoming, and several National Forests in the Rocky Mountains region each drafted an Environmental Statement and a benefit/cost analysis for a multiyear control program of the mountain pine beetle. Most certainly future control programs will follow suit.

The Environmental Statement

The preparation of an Environmental Statement (ES) has proved an effective and necessary tool in the Northeast for exploring the impacts of gypsy moth control-versus-no-control options. The ES guides an agency proposing a control program in discussing the ecological and socioeconomic impacts of target organisms; setting priority areas to be controlled, thereby describing an explicit definition of impact and the way in which it will be measured; discussing alternate ways of control and their impacts; formulating a benefit/cost analysis of the proposed action; and providing a public forum through which interested individuals or groups can voice their opinions.

Ecological impacts of the gypsy moth have always been easier to measure than socioeconomic impacts. The ES prepared for the 1973 gypsy moth suppression program indicates that "there is considerable information on tree mortality, lost growth in trees, and changes in forest stand composition, but less information on impacts on birds, fish, other wildlife, soil, water, . . . and on people" (U.S. Department of Agriculture et al. 1973). This early document analyzes in great detail the effects of gypsy moth on the ecological considerations; however, the socioeconomic impacts on recreation, real estate value, environmental amelioration, and the like are speculative at best. This is unfortunate because the 1973 document indicates that the "States have been strongly encouraged by certain sectors of the public to treat the gypsy moth in selected areas" (U.S. Department of Agriculture et al. 1973).

In 1973, New Jersey designated areas for gypsy moth control in the following order or priority: Forested communities with at least 20 homes per 40 ha, and State recreational areas; municipal and county recreational areas; forested communities with 5 to 19 homes per 40 ha; and watershed areas.

Similarly, New York proposed that these areas receive priority treatment: Recreation and special use areas, forested communities, and high-value forests. Pennsylvania and Rhode Island, with minor modifications, had the same priorities (U.S. Department of

Agriculture et al. 1973). The selection of priority areas in the 1973 Environmental Statement exhibits how public response can influence management objectives. This further reinforces the importance of the need for better measurement of the socioeconomic impacts created by the insect.

Five years later in the Final Environmental Statement for Cooperative Gypsy Moth Suppression and Regulatory Programs, 1978 activities, the priority areas set by New Jersey and Pennsylvania (the only two States proposing to suppress the gypsy moth) were still the same (U.S. Department of Agriculture et al. 1978). However, by this time progress had been made in methods for determining the economic importance of gypsy moth effects on wood production, real estate, recreation, wildlife, water quality, and other forest resources.

Once the impacts of gypsy moth were defined in the Environmental Statement, the next step was to assess the relative benefits of the control-versus-no-control option. This was accomplished through the use of a benefit/cost analysis.

Benefit/Cost Analysis

The development of benefit/cost analysis and its subsequent use is not new—it has its origin in the early 1900's (Hammond 1966). However, the application of the concept in assessing the benefits of forest pest control programs, in particular the gypsy moth program, is unique. As with any new application of an evaluation technique, problems exist with establishing the proper assumptions and base data needed in order to make prudent assessments. One of the serious pitfalls associated with a benefit/cost analysis is the assumption by those preparing the statement that the benefits they perceive as primary are viewed the same way by the public (the I-know-what-is-best-for-you-attitude). Fortunately, there are provisions made in the Environmental Statement to allow for outside comment on both the assumptions made and the subsequent benefit/cost analysis. For example, the Environmental Defense Fund responded to Pennsylvania's Environmental Statement for gypsy

moth suppression programs in 1972 by stating: "The Pennsylvania Department of Environmental Resources makes no cost-benefit analysis as such, but it makes a convincing case for the gypsy moth to be controlled because of the extent of public demand. . . . However, the statement does not indicate that there might also have been public opposition to the control program." The Environmental Defense Fund concluded their comments with, "we hope that next year's statement will have a more thorough cost-benefit analysis" (U.S. Department of Agriculture et al. 1972). Indeed, in the Environmental Statement submitted in 1973, the Pennsylvania Department of Environmental Resources attempted to quantify the effects of gypsy moths on high-value forest stands, real estate values, lost income from tourism, and lost recreation value (U.S. Department of Agriculture et al. 1973).

Traditionally, the value of trees as a timber resource has often been the only impact readily quantifiable in benefit/cost analyses. This is because the ecological impacts of forest pests, such as tree mortality and growth loss, are easily measured and lend themselves to marketplace evaluation. For example, in 1976 New Jersey developed a benefit/cost analysis for gypsy moth control. Among the objectives were reducing the nuisance factor of the caterpillars; minimizing frass pollution in streams, lakes and ponds; and preventing defoliation in excess of 30 percent. However, the only benefit that the State could quantify in the analysis was the timber-resource value. The intangible benefits for which there were no assessments made were: Maintaining a favorable environment for wildlife, preserving water quality, preserving esthetics, preserving the recreation value of State forests and parks, and preventing an increased fire hazard (New Jersey Bureau of Forestry 1976).

Gypsy moth nuisance and damage costs are difficult data to estimate because they primarily involve personal utilities not directly subject to marketplace evaluation. These considerations are not within the traditional realm of forest entomology, and the need to involve people from other scientific disciplines, including economists and sociologists, is

apparent. Gypsy moth impacts on people are among the most important, the most difficult to measure, and are, without doubt, the most dynamic because public opinion and human tolerance for the insect are variable and based on individual judgments. The challenge facing scientists was to develop and expand on existing techniques and data for making sound assessments of gypsy moth impacts on these more esoteric values.

Postcontrol Evaluation

There are few long-term investigations designed to show differences in gypsy moth populations and their impact in treated and untreated areas. Such information is essential before management strategies can be developed. The use of postcontrol evaluation is a powerful tool for measuring this vital data and can also be used to gauge how many of the long-term benefits of the control program were actually captured.

As an example of this kind of evaluation, in 1971 the Forest Service initiated a postcontrol evaluation of the efficacy of the pesticide Sevin 4-Oil® in reducing gypsy moth populations, tree defoliation and tree damage caused by defoliation, and to accumulate data to test and possibly modify predictive models for estimating future gypsy moth populations and subsequent damage (Terry and Bridgewater 1978). Five years after treatment a significant difference was recorded between treated and untreated areas for each of the following parameters: Tree mortality, tree volume, tree value loss and tree radial growth loss. This indicates that Sevin 4-Oil® is effective against the gypsy moth and that long-term benefits can be realized from a single application of Sevin 4-Oil®.

Forest Stand Hazard Rating

Research within the program was directed at developing systems for rating forest stands as to their susceptibility and vulnerability to the gypsy moth. Houston and Valentine (1977) developed a system whereby stand susceptibility can be assessed by measuring certain structural-feature variables of trees—bark flaps, bark fissures, branch stubs, and others—separately for tree species in different gypsy moth food-preference classes. This study is discussed further in chapter 5 and is currently being evaluated.

Two other prediction techniques show promise after initial evaluations. Both were developed from data collected from 1970 to 1976 on 0.04-ha sample field plots in Pike and Monroe Counties, Pa. Herrick et al. (1979) applied the automatic interaction detection (AID) technique to diagram tree loss and timber value loss in order to identify those combinations of stand conditions that are likely to sustain high losses. This information will assist in identifying high-risk stands as candidates for gypsy moth control measures.

Gansner et al. (1978) developed equations for rating the potential hazard of impending gypsy moth attacks to forest stands. The equations estimate expected tree mortality in terms of the following easy-to-measure key elements of forest stand conditions:

$$HRN = 11.85 + 0.82(NPC) + 0.0005(NWO)^2$$

$$R^2 = 0.73$$

$$HRP = 4.16 + 0.83(PPC) + 0.001(PWO)^2$$

$$R^2 = 0.57$$

where

HRN = Number of trees per acre that will die.
HRP = Percent of trees that will die.
NPC = Number of live trees per 0.4-ha with poor crowns.
NWO = Number of live trees per 0.4-ha in the white oak species group.
PPC = Percent of live trees with poor crowns.
PWO = Percent of live trees in the white oak species group.

The poor crown condition class was assigned to trees with crowns consisting of 50 percent or more dead branches (allowances permitted for non-self-pruning species); foliage density, size, and coloration of definite subnormal quality; and heavy epicormic sprouting.

The value of a forest stand hazard rating system to pest managers is obvious. If one or more of these three techniques prove workable then pest managers will be able to reliably assess long-term socioeconomic impacts of the gypsy moth and allocate resources accordingly.

The Northeastern Area State and Private Forestry, Forest Insect and Disease Management group of the Forest Service is currently conducting a 5-year study on over 600 plots in central Pennsylvania to evaluate these procedures.

Effects of Defoliation

Because insect-caused tree mortality is readily observed, this variable is typically used as the basis for assessing the socioeconomic impact of forest insects on forest values. In the case of some insects, such as bark beetles, tree mortality occurs between the time that the insects attack the tree and the time that the new brood emerges. It is therefore a simple task to tabulate the amount of tree mortality and make an assessment of the impact on other forest values. The difficulty in assessing the overall socioeconomic impact of gypsy moth arises because no distinct line exists between the consequence of insect attack—in this case defoliation—and tree mortality, as there is with bark beetles.

The long-term effects of repeated tree defoliation by gypsy moth are well documented and discussed in chapter 5. Earlier researchers suggested that defoliation may actually accelerate natural forest succession resulting in less susceptible forest stands (Bess et al. 1947). Houston and Valentine (1977) also suggested that as a consequence of high oak mortality, some stands may be more resistant than before. Campbell and Sloan (1977) believe that this can possibly be attributed to selection within any given species for trees that are less prone to defoliation and to changes in stand composition. Collins (1961) indicated that understory trees exhibit an increased growth rate when the overstory is defoliated. If this provides benefits to wildlife, as many today believe, then it should be considered when assessing the total impact of gypsy moth defoliation.

Predicting the short-term effect of defoliation on subsequent tree mortality has been the most difficult relationship to establish—unfortunately it is also the most critical information needed to assess the socioeconomic impacts of gypsy moth. The problem exists because the ultimate effect of single or multiple defoliation depends upon the condition of the tree prior to being defoliated. This assessment is seldom available. Additionally, tree mortality is not always coincident with defoliation and may occur 3 to 5 years after the trees are first defoliated. This results because most tree mortality is eventually caused by opportunistic organisms that attack trees after they are weakened by defoliation.

Kulman (1971) reviewed the effects of insect defoliation on tree growth and mortality and compiled an extensive array of defoliation, growth loss, and mortality figures for many defoliating insects. He also indicated that because many factors influence defoliation and subsequent mortality, consideration of these other physiological factors should improve the sensitivity of growth and defoliation measurements. Soon thereafter, Campbell and Valentine (1972) published a series of tables addressing tree degradation and mortality attributable to gypsy moth defoliation. With this quantitative tool in hand, it became possible to develop the rudimentary methodologies by which to assess the socioeconomic impact of gypsy moth on such values as timber, residential property, ownership objectives, and others.

Values at Risk

Prior to the Expanded Gypsy Moth Program it was apparent that more base data and new procedures were needed in order to assess the overall impact of gypsy moth. Six resources, or values at risk, were identified—timber, residential property, ownership objectives, fire hazard, wildlife, and watersheds—and subsequent investigations were carried out prior to and during the Expanded Gypsy Moth Program.

As a result of the progress made in estimating gypsy moth caused tree degradation and mortality, in 1972 the Northeastern Area, State and Private Forestry,

and Northeastern Forest Experiment Station cooperatively initiated a new station publication series, "Economic Analysis of the Gypsy Moth Problem in the Northeast." The series intended to provide land managers of various disciplines with procedures for making economic impact estimates relative to gypsy moth defoliation. Presumably, land managers such as State personnel, resort owners, timber producers, park officials, and homeowners will find one or more of the publications directed to their need for making more informed gypsy moth control decisions.

The principle that was applied in the evaluation is in the form of an equation:

Value of gypsy moth control equals (1) losses incurred if gypsy moth is unchecked, plus (2) environmental risks and uncertainties if gypsy moth is unchecked, minus (3) direct control costs minus environmental control risks and uncertainties.

Currently in print in the series are the socioeconomic impacts of gypsy moth on three resource areas—timber, residential property, and ownership objectives; a brief discussion of each follows. The remaining three subject areas—fire hazard, wildlife, and watersheds—are currently being reviewed for incorporation into the economic series. The discussion of these last three resource areas will be in much greater detail, as some of the new base data and methodology will be presented.

Timber

The first publication in the economic series presents an approach for evaluating timber product loss following gypsy moth defoliation (McCay and White 1973).

To develop information on stand value loss, certain basic information was required: Time dimension, extent of damage, and stand value. These parameters were integrated into models for evaluating local opportunities for gypsy moth control using appropriate economic inputs. The methodology is then demonstrated using case studies of gypsy moth infestations in pulpwood and sawtimber-sized stands for both short- and long-run analyses.

Extent of damage in a forest stand as it relates to gypsy moth defoliation is one of the most difficult parameters from which to obtain estimates in support of an economic analysis. The expected tree mortality rates used by McCay and White were obtained from Campbell and Valentine (1972). In the last 5 years, it has become apparent that these tables, developed from data collected in old established infestations in New England, were not performing well in the more recent areas of infestatation in Pennsylvania, New York, and New Jersey. It is anticipated that more reliable timber degradation data will result from the field testing of the new gypsy moth prediction models, discussed earlier in this chapter.

Residential Property

The second paper in the economic series presents guidelines for determining dollar losses in residential property values from tree mortality caused by the gypsy moth (Payne et al. 1973). To utilize this technique in a residential community, it is necessary to know property values, lot sizes, and the number of trees 15.4 cm in diameter at breast height (d.b.h.) or larger per lot, for a sample of residential properties.

In an earlier study in Massachusetts, Payne (1971) evaluated the contribution of trees to property values. In that study, trees contributed an average of 7 percent and as much as 15 percent to the value of $25,000 to $50,000 homes on lots averaging 1,840 m^2. The relationship of values contributed by trees (V) to the number of trees 15.4 cm d.b.h. and larger (t) has the following form:

$$V = 300t - 5.22t^2 \quad \text{for } 0 < t < 50$$

and

$$V = 1950 \quad \text{for } t \geqslant 50$$

and results in the generated values shown in table 7–1.

Time and manpower limitations prevented a replication of the earlier Massachusetts study, thus it was assumed that the equations were also applicable in Stroudsburg, Pa., where the current study was performed.

Table 7-1.—*Contribution to residential property value from trees 15.4 cm d.b.h. and larger*

Number of trees	Dollar value	Number of trees	Dollar value	Number of trees	Dollar value
0	$ 0	17	$3,590	34	$4,160
1	290	18	3,710	35	4,100
2	580	19	3,810	36	4,030
3	850	20	3,910	37	3,950
4	1,120	21	4,000	38	3,860
5	1,370	22	4,070	39	3,760
6	1,610	23	4,140	40	3,650
7	1,840	24	4,190	41	3,520
8	2,070	25	4,240	42	3,390
9	2,280	26	4,270	43	3,250
10	2,480	27	4,290	44	3,090
11	2,670	28	4,310	45	2,930
12	2,850	29	4,310	46	2,750
13	3,020	30	4,300	47	2,570
14	3,180	31	4,280	48	2,370
15	3,320	32	4,250	49	2,160
16	3,460	33	4,210	50	1,950

Source: Payne et al. 1973.

Figure 7-1 depicts in a photo matrix the range of residencies, in terms of property value and number of trees, that should be included in any sampling of a forested residential community. These sample residencies can then be evaluated as to current value by local real estate appraisers.

The key to employing this technique is having available predictions of tree mortality from a gypsy moth attack. Like the commercial forest stand study, the mortality rates used in this study were obtained from Campbell and Valentine's (1972) tree condition and mortality tables. The limitations of these tables as expressed earlier restrict the use of this technique until more reliable estimations of tree mortality can be developed.

Ownership Objectives

The third paper in the economic series presents a regional approach in quantifying gypsy moth related effects such as nuisance, defoliation, and tree mortality, and their impacts as they relate to recreational and esthetic values of forest land and forested communities (Moeller et al. 1977). A subsequent study by Marler and McCrea (1977) attempts a more localized approach utilizing the same data as Moeller et al.

The methodologies and presentation of results are quite rigorous and too complex to attempt to outline here; the reader is referred to the cited publication. However, four major areas of investigation—impacts on objectives, control costs, financial losses, and recreational losses—are summarized below for homeowners, because the attitudes and actions of this group help to place the gypsy moth problem into perspective.

• The principal impacts on homeowner objectives for gypsy moth related effects of nuisance and defoliation were on backyard recreation and on the enjoyment of natural beauty. Tree mortality ranked a distant third. Three factors might tend to increase a homeowner's concern for tree mortality: Increased mortality on property, education to the probable consequences of defoliation, and extended familiarity with the gypsy moth and its effects—that is, having experienced the effects of gypsy moth over time, the homeowner can place them in a more rational perspective.

Figure 7-1—*Matrix of residences from forested communities showing ranges of property value (y axis) and amount of forested cover (x axis) that should be considered when sampling a community.*

• From the sample group, homeowners who participated in a cooperative public control program (250 respondents) spent an average of $62, of which $16 went for expenses other than their own time. In contrast, homeowners who paid commercial control measures (32 respondents) spent an average of $146, of which $120 went for expenses other than their own time.

These data are further supported by a survey conducted in New Jersey (Kegg 1976). In this survey of Monmouth County, costs of treatment, both public and commercial, including the homeowners own time, are quite similar to the results of Moeller et al. (1977).

• The financial losses attributed to gypsy moth consisted of capital costs incurred in coping with the infestations plus increases in maintenance costs, reduction in property values, and, where applicable, reduction in revenues.

Homeowners who paid for commercial control (24 respondents) sustained an average financial loss of $292, in contrast to the average loss of $76 sustained by the homeowner who participated in public control (202 respondents).

• Of the 59 respondents who paid for commercial control, 44 percent lost an annual average of 133 person-days of recreational use of their property because of the gypsy moth infestation. Of 481 respondents in the public cooperative control group, 51 percent lost an annual average of 108 person-days of recreational use of their property.

In summary, it should be emphasized that for all ownership groups it was demonstrated that specific ownership or management objectives were seriously affected by a gypsy moth outbreak.

Fire Hazard

The effect that gypsy moth has on forests to increase the fire hazard is one impact that is rarely not mentioned in benefit/cost analyses, yet it always escapes assessment. In New Jersey's 1976 benefit/cost analysis, the reduction of fire danger, attributed to a control option, was grouped among several other items in a category called "intangible benefits." The

State indicated that "severe defoliation resulting from gypsy moth causes increased fire danger. It opens the canopy exposing the litter and understory to direct sunlight, thus accelerating drying and making that litter more susceptible to ignition, and increased intensity of wild fire . . . . The foregoing benefit(s) derived from the preceding discussion(s) (is) largely intangible and at present the formula for attaching monetary values to these is wanting" (New Jersey Bureau of Forestry 1976).

It seems plausible that forest defoliation by the gypsy moth can alter the forest microclimate and fuel load, resulting in an increased fire hazard. A technique has been developed for placing a dollar value on this increased fire hazard that utilizes the National Fire-Danger Rating (NFDR) system, which measures the potentials for forest fire occurrence and severity, given a set of parameters (Deeming et al. 1974). The NFDR system is composed of three indices: Occurrence, burning, and fire load. The occurrence index is derived from risk, or the degree to which an area will be exposed to ignition from lightning and man, and the ignition component, or the potential for a spreading fire to occur if a burning cinder (firebrand) is introduced into fire fuels. The burning index is derived from the spread component, or the rate at which the fire front moves, and the energy release component, or the rate of combustion. Lastly, the fire load index is a measure of the total amount of effort required to contain all probable fires occurring within a rating area during a specified period.

The effects that defoliation has on the forest microclimate increase the occurrence, burning, and fire load indices. Without the natural cover provided by foliage, the forest floor is subject to greater insolation. The temperature of the microclimate rises while the relative humidity and the fire fuel moisture decrease. Larger forest materials dry at a faster rate. All of these serve to increase the potential fire hazard.

In using the NFDR system as the basis for assessing fire hazard, three fire-related costs can be computed—fire prevention, fire treatment, and fire damage. Because the fire load index (FLI) is defined in terms of the effort necessary to contain a fire, it can be assumed

that each index unit of FLI is convertible to expected dollars of treatment and prevention costs. In the same fashion, expected damage costs (DC) per index unit of FLI for each resource type should be derivable. Given these expected cost figures in terms of index units of FLI, the total expected cost of a fire can be computed (table 7-2) and the difference in forest fire hazard costs between a defoliated and undefoliated area can be readily determined.

It is important to realize that these equations deal with expected levels. The fire load index for a defoliated forest (FLI$_D$) is an expected, or average, value. Expected values are important for planning purposes. To determine where to concentrate firefighting equipment, it is important to know the relative expected potential occurrence of a fire and the value threatened. This computation is dependent upon the ability to assess fire damage to such values as a watershed, real and personal property, timber, recreation, wildlife, and forage resources.

The NFDR system provides the tool with which to determine the potential, or expected, occurrence and severity of forest fires. Once information becomes available on the expected costs of damage per index unit, then the cost of a forest fire hazard and the increased cost due to defoliation by the gypsy moth can be determined.

Table 7-2.—*Computation of the cost of a forest fire hazard*

FLI$_D$ = expected index level for a defoliated forest
FLI = expected index level for an undefoliated forest
C = total cost of a forest fire hazard, dollars
C$_D$ = total cost of a forest fire hazard for a defoliated forest, dollars
PC = prevention cost, dollars/index unit FLI (if allowed to vary)
TC = treatment cost, dollars/index unit FLI
DC$_T$ = for a timber resource
DC$_W$ = for a watershed
DC$_R$ = for a recreation resource
DC$_L$ = for a wildlife resource
DC$_F$ = for a forage resource
DC$_P$ = for real and personal property
C = FLI(PC + TC + DC)
C$_D$ = FLI$_D$(PC + TC + DC)
C = additional cost of forest fire hazards due to gypsy moth defoliation = (FLI$_D$ - FLI) (PC + TC + DC)

Source: Moore et al. 1978.

Wildlife

The effects of tree defoliation and subsequent mortality on wildlife are other topics that generally receive unsubstantiated attention in environmental statements. The value of wildlife is a difficult resource to assess, mainly because there are many ways to approach the subject. A study by Moore et al. (1978) suggests that the most straightforward manner in which to determine the possible benefits of preventing the decrease in wildlife in a given area is to consider to whom it is valuable—that is, who would pay to have wildlife preserved. Because the impacts of gypsy moth directly affect *people,* Moore et al. assumed that the primary economic importance of wildlife is recreational—hunting, fishing, and birdwatching. The methodology utilized by Moore et al. is an adaptation of a set of equations developed by Unger (1976).

The basis of this approach is that the benefits of gypsy moth control can be viewed as a function of the increase in area available for hunting, fishing, and birdwatching, and in the number of recreation days. Presumably, if the gypsy moth is controlled, the number of suitable hunting and fishing areas will increase. This should cause a subsequent decrease in the cost of transportation and time (transfer cost) to get to such an area, and consequently more people will take advantage of the lower cost. This approach lends itself nicely to traditional marketplace evaluation, but there are difficulties associated with this type of analysis when studying gypsy moths.

If the effects of the moths on wildlife occur only during the period of defoliation, then monthly data would be appropriate. Also, if hunting and fishing seasons do not occur during the period of defoliation, then there is no loss of wildlife benefits, regardless of whether the wildlife moves out of such areas or not. However, if the effects of gypsy moth are permanent, then excessive tree mortality could cause habitat conversion and wildlife species adjustment. Such an occurrence could be regarded as either beneficial or detrimental, according to the wildlife species involved, and points out the need for setting wildlife priorities before making a benefit/cost analysis. For example, if, from the hunter's point of view, whitetail deer

populations are of prime interest, then any change in habitat caused by the gypsy moth that results in reducing the deer herd is detrimental. On the other hand, if another wildlife species is preferred over deer, then such a change in habitat might be beneficial. The model also fails to take into account the esthetic value of wildlife and endangered species and certainly has other deficiencies, too.

Undoubtedly, no model will ever become sophisticated to the point where everyone's questions or concerns about wildlife will be answered. Wildlife is such a broad area, covering everything from large game animals to small birds, that someone's special interest will always be threatened. Nevertheless, with these weaknesses kept in mind, a careful application of this model should yield a reasonable, although somewhat restricted, estimate of the costs of gypsy moth effects on wildlife resources.

Watersheds

As was the case of the previously discussed values at risk, the potential effects of gypsy moth on watersheds, in particular water quality and quantity, are not well known. Progress has recently been made in developing necessary base data and models for assessing the impact of gypsy moth on water quality and quantity.

The costs of impaired water quality were investigated in three primary areas: Nonresident recreation, property values, and water treatment for consumption. Other areas such as esthetics and commercial fishing were also considered. The costs or benefits of water-quantity changes were a function of fluctuations in watershed output. A more thorough discussion concerning the development and application of these models can be found in the base study by Moore et al. (1978).

Assessing the impact of defoliation on water quantity is a simple task. Once the calculated cost of water is known, this figure can be multiplied by the change in water output by a watershed. In New Jersey, for example, 75 percent defoliation in the Newark watershed resulted in an *increased* water output of 1,365,000 l per hectare (New Jersey Bureau

of Forestry 1976)—about a $7.93 per hectare benefit that can be attributed to defoliation. It is clear from the literature, and from actual events such as the Newark watershed, that gypsy moth defoliation will increase stream flow, primarily in the growing season, and that the effect can be expected to last up to 10 years. For this period of time, then, there is actually a benefit of increased water due to defoliation. However, pine trees often follow oaks in areas of oak mortality, and pines have higher evapotranspiration rates. In the long run, watershed output could actually be reduced (Moore et al. 1978). Therefore, an assessment of this potential decrease in watershed output would be in order.

The base data for water quality are still inconclusive. Studies made in clearcut areas speculate that defoliation along streams could cause a rise in water temperature. However, in central Pennsylvania, the habit of gypsy moth to infest ridgetops rather than valleys where streams occur tends to minimize this impact. Even if the gypsy moth defoliates trees along a stream, the stems and branches would still provide about 50 percent cover over the stream. Other studies indicate that defoliation has no effect on stream turbidity (Moore et al. 1978).

The effect of gypsy moth defoliation and frass droppings on streamwater nutrient levels is another area of concern. It has been suggested that defoliation might increase the rate of decomposition of forest litter, and the excess nutrients might leave the watershed and pollute streams. However, studies made in clearcut areas did not indicate a reduction in water quality. Thus the nutrient increases for gypsy moth defoliation should remain well within drinking water standards (Moore et al. 1978).

Additional water-quality data, available from an intensive 2-year study by Moulding (1977) on the impact of gypsy moth frass on stream nutrient loading, indicate that it is very unlikely that frass phosphorus would be exported from watersheds via soil percolate and base flow. This leaves storm surface runoff and subsoil runoff as the only paths available for export. The amount of runoff and hence the amount of phosphorous transported would depend principally upon duration and intensity of the storm event,

percent slope, permeability of the soil, and antecedent soil moisture conditions. Moulding calculated that the nutrient content of the frass on his study site contained only one-half the nitrogen and one-thirtieth of the phosphorous that is typically applied as fertilizer to crops in New Jersey. Unfortunately, during the study defoliation did not materialize to the degree necessary to allow an empirical determination of possible eutrophication effects of any frass nutrients transported to streams. Moulding speculates, however, that in other geographical areas with steeper slopes, less permeable soils, surface bedrock, etc., frass might more easily be transported to streams and add to nutrient loading. This would likely show up in stream stormflow and require sophisticated sampling techniques to be quantified in the stream.

The studies by Moulding (1977) and Moore et al. (1978) provide some of the necessary base data for describing water quality and quantity impacts, which have been lacking in the past. However, much more work is needed to expand the existing base data so that the described methodology can be evaluated.

Summary

Historically, little time or effort has been given to assessing the socioeconomic and environmental impacts of the gypsy moth or its control. However, the general public of today, which is generally better educated and informed, is genuinely interested in environmental issues and demands a forum in which to voice opinions. This opportunity was provided by Congress in 1974 through passage of the National Environmental Protection Act (NEPA). The Environmental Statement (ES) and its review process, as required by the NEPA, elucidates the effects and impacts caused by the gypsy moth and lists the alternative controls (including no control); it also provides a valuable mechanism for public response. When the impacts caused by gypsy moth are defined, the benefit/cost analysis is used to place a dollar value on the cost of the control option chosen and its resulting benefits. It is this latter category—benefits—that is the most difficult to assess. The Expanded

Gypsy Moth Program attempted to hasten development of methodologies for assessing the socioeconomic impacts of gypsy moth, and thereby facilitate valuation of the benefits of control programs.

As already pointed out, one of the biggest missing pieces to the impact assessment puzzle is information on expected tree mortality following a regime of gypsy moth defoliation. This significant shortcoming may be remedied or at least moderated with the development of the previously discussed risk-rating procedures (Gansner et al. 1978, Herrick et al. 1979, Houston and Valentine 1977).

Upon completion of the publication series, "Economic Analysis of the Gypsy Moth Problem in the Northeast," land managers will have at their disposal an array of tools to assist them in evaluating the potential impacts of gypsy moth upon their forest resources. In some studies the land manager will find impact information that can be utilized directly in evaluating alternative management decisions. For other areas of concern, one or more of the impact assessment tools may need to be applied to the forest resource.

Each of the studies on fire hazard, wildlife, and watersheds (water quality and quantity) developed a method by which to calculate the potential damage caused by the gypsy moth and provided base data that have been lacking in the past. However, as these base data and subsequent models evolved, it became obvious that more questions needed to be answered. In the case of fire hazard, for example, a model now exists for assessing damage, yet it remains to be seen whether or not increased fire potential is really a primary cost in the Northeast. Similarly, the base data for wildlife and watershed resources need to be expanded.

The assessment chore is by no means complete, even though some guidelines exist with which to work. As these methods are employed, an impact data base will accumulate and new techniques will emerge. Ultimately, these tools and data should be consolidated into a socioeconomic model that can assist the decisionmaker. The key to success for these various impact assessment techniques will be how

their individual results are balanced one with the other in answering the wealth of resource protection questions relative to the onslaught and aftermath of a gypsy moth infestation.

One possible unifying mechanism may be the integration of these tools into the Comprehensive Gypsy Moth Management System (chapter 8). This system will attempt to provide a framework for the coordination of all ongoing gypsy moth related activities such as research, applications, field operations, and resource allocation. Since the effects of gypsy moth have a great impact on people, the actual decisionmaking process for control or no control options will be left up to the local citizenry through their respective State, county, and municipal governments.

As we work toward the gypsy moth management system, the impact models should be tested and of course applied in as many situations as needed and possible. Perhaps then they can be expanded so as to have regional applicability.

The Expanded Gypsy Moth Program supported the continued development of procedures and related information relative to assessing impacts of a gypsy moth infestation. The greatest challenges are yet to be faced, and the rewards will be realized in the future after the tools are integrated and functioning. At this future point in time, scientists will be able to place the gypsy moth with its related effects and subsequent impacts into proper perspective. Land managers can then dispatch their assigned duties without the demand for immediate and questionable solutions to the gypsy moth problem.

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Pest Management Systems Development

8

Daniel O. Etter, Jr.

Introduction

A major effort is being devoted to the detailed planning of a comprehensive gypsy moth pest management system that covers all aspects of the problem and includes specific assignment of responsibilities to specific organizations and Federal and State agencies. The goal of this systems-planning work is the implementation of the most effective operational system that can be devised.

The concept of a pest management system always refers to a system that includes smaller subsystems and that is itself part of some larger systems. There is no "correct" way to define the system boundaries, and no compelling need exists at this time to make an arbitrary selection. In a general way, a pest management system is understood to include whatever is needed to utilize available resources with the goal of alleviating the adverse impact of the pest. However, no exact, concise definition of a pest management system is proposed in this chapter.

To avoid misunderstanding and possible misinterpretation, it seems necessary to state explicitly how some terminology is used in this chapter.

A *pest management tactic* is a particular, detailed decision—for example, the decision to spray a particular block with 1 kg per ha of Sevin 4-Oil® from fixed-wing aircraft using flat fan nozzles (more or less detail might be necessary depending upon the purpose of the specification). It is recognized that, in the context of pest management, the term *control* usually refers to suppression and, in addition, usually to the use of a pesticide. Control is sometimes used in its broader meaning in this chapter and should not be automatically equated with suppression.

In this chapter, *integrated control* and *integrated pest management* are understood to mean the same thing. Integrated pest management is characterized by the use of certain criteria in the selection of pest management decisions which are formulated in this chapter as follows (Doutt and Smith 1971):

• Use pest management tactics that resemble natural biological or ecological responses as closely as

possible (examples include the selection of resistant tree strains and species and augmentation of natural enemies, including microbials).

• Adapt pesticide applications as closely as possible to the minimal requirement for the actual field conditions.

• Allow natural regulating mechanisms to counteract undesirable pest/stand trends to the greatest degree possible.

It is understood that these criteria are used in conjunction with more conventional benefit/cost criteria based on direct costs of tactics and risks of damage.

Five major problem areas are listed together under the general heading of *systems-related efforts:*

• Data management (data preparation, keypunching, and editing and update of files).
• Data analysis.
• Development of operational forecasting methods.
• Development of simulation models for long-range systems planning and design.
• Planning and design of a gypsy moth pest management system.

All of these areas received some attention before the Expanded Gypsy Moth Program was initiated, but most of the effort was devoted to the first three areas. The increased funding provided by the program made it possible to expand the scope of the efforts into the last two areas.

The first major milestone in the expanded systems effort was the publication of the results of a preliminary design study by Ketron, Inc.; the purpose of this study was to develop in detail a comprehensive overview of the full range of problems associated with management of the gypsy moth in the United States (Blacksten et al. 1977). The Ketron study established the urgency of incorporating an organizational viewpoint into the systems effort. It also identified some major areas requiring analytical effort. These areas included an analysis of the benefits and costs of gypsy moth containment activities, the preliminary design of a reporting system, and an associated analysis of the use of data concerning overall systems operations. These studies are nearing completion, and

the results will be used to assess the relative merits of alternative pest management system designs.

This chapter contains discussions of the design of a comprehensive gypsy moth pest management system and the use of models of various kinds. The discussion of system design treats both the approach and the results to date of the system design effort. Organizational considerations and comprehensiveness of scope have been emphasized in the design work. The section on modeling assumes some systems analysis background and is intended to show how models are related both to the design and to the operational pest management system.

Comprehensive System Design

The approach to improving gypsy moth pest management taken here uses a concept of system found to be particularly fruitful in planning and engineering projects. The choice was made specifically to facilitate implementation of those results that promise a real improvement in pest management practices. Experience in the field of operations research and management science has been that relatively few of the models developed and described in the literature are ever used in real decision situations. Powell (1976) presents a convenient summary of some experiences, conclusions, and conjectures concerning implementation of project findings or models. He quotes the results of a study by Urban (1974), indicating that some 3 percent of the models published over a 30-month period were put into production (repeated routine) use, and only 15 percent were used in real decisionmaking at least once. There is no obvious reason to expect a higher probability of successful implementation of research models for gypsy moth pest management.

The inference is that a substantial effort is generally needed to translate research findings, and modeling and analytical results especially, into visible improvements in practice. This is particularly important if the translation involves an integration of a variety of findings into a coherent whole (a new or improved "system").

Systems Planning and Design

Approach

Nadler (1970) developed and described an approach to systems planning and design that appears to be particularly effective. It is called the Purposes-Target-Results approach. Nutt (1976, 1977) has conducted an experimental comparison of Nadler's method with some other commonly attempted methods in the context of planning problems in the health sciences field. The plans produced were compared and rated by outside experts, administrators, and nursing staff. It was concluded that Nadler's method led to significantly higher quality plans. Some other comparisons are presented by Nadler (1977), Schultz et al. (1976), and Friedman (1973). Some aspects of Nadler's approach are discussed below.

From a purely descriptive organizational viewpoint, a system can be characterized roughly as follows. It is the complex of people, equipment, procedures, facilities, management controls, and plans needed to utilize available resources to achieve some specific organizational purposes. This viewpoint emphasizes that the systems of concern are real, dynamic entities. They are intrinsic and essential to the day-to-day functioning of real organizations. They may work well or poorly, but they are real and are not separable from the organization, its purposes, and the resources it uses.

Another viewpoint, prescriptive in nature, is the one that is naturally used for planning and systems design work. Every system has certain elements, each of which has a set of specifications or "dimensions." One of Nadler's major contributions has been a codification of system elements and sets of specifications that must be treated in order to have a high degree of assurance that system plans or designs will be realistic and readily capable of implementation; in fact, Nadler identifies implementation as a major design step that must be accomplished before the design task can be considered to be complete. This step is not always recognized as part of planning or design; the omission of implementation and any

necessary subsequent revision or debugging as a crucial part of the problem seems to be a major reason for the low rate of successful implementation of analyses and modeling projects.

Successful implementation is the real criterion for assessing the values of an analysis, plan, or system design or improvement. However, implementation is a management prerogative. A consultant or analyst has no capability to enforce adoption of recommendations. One conceivable inference is that results must somehow be sold to the manager. Nadler suggests an alternative—that the manager must solve the problem himself. The problem of the system planner or designer is to remove most of the burden of this task from the manager by creating a situation in which the experience and objectives of the manager are used and amplified by the appropriate technical expertise to arrive at a solution satisfactory to the manager. The task is therefore to overcome the problems encountered by the manager in formulating a practical solution. The detailed part of Nadler's approach presents methods for accomplishing this, and these methods form a crucial portion of the overall attack upon systems planning and development problems.

Comparison with Other Forest Pest Management System Projects

Efforts to develop forest pest management systems have generally followed the patterns proposed by Watt (1961, 1963, 1964). Emphasis has been placed on three major areas, with the development of a system of computerized models as the central and integrating objective. The models are intended to simulate, or predict, the pest population dynamics, stand response, treatment effects, and socioeconomic impacts. The other two main areas are the sampling and survey methods that are needed to provide the field data for the models and the possible improvements in agents or other treatment or control measures. A substantial part of the gypsy moth effort is directed toward the development of predictive models, sampling and survey methods, and control measures.

The spruce budworm model (Holling, et al. 1976) provides an interesting and elaborate example of such a system. The practical, operational objective of the effort will provide a means for testing somewhat simplified or idealized pest management policies in order to develop operationally useful policies. In this context, a policy should be understood as a rule that specifies a management tactic as a function of the pest population/ stand conditions. The system of models is thus an instrument for facilitating the activities of pest managers (or their staffs) directed toward the improvement of pest management practices.

The Douglas-fir tussock moth program (Wright 1977, Campbell and McFadden 1977) has as one of its objectives the development of models that would permit similar explorations for policy development work. Campbell's discussion of the integration problem of the Douglas-fir tussock moth program (Campbell 1977) suggests the goal of making the system of models real-time decision aids, not only for pest management operations planning but also for continued system improvement (Cambell 1973).

The discussion of the lodgepole pine/ mountain pine beetle system development work by Anderson et al. (1976) refers explicitly to the integration of impact models, population and stand models, and strategy analysis systems as a pest management system. They seem to propose the goal of deriving analytically a prescription, or policy, for pest managment decisions that is at least very close to that which the forest manager could implement directly.

The outlook for the regular use of models for planning in these cases is relatively good. Large forest tracts are already being managed with specified and uniform objectives, and extensive use of stand models and other computerized resource planning aids is already standard practice. Moreover, unlike the gypsy moth, the pests are native to North America. The pest population dynamics can be expected to be relatively homogeneous, as can the host/ pest interaction. In the case of the gypsy moth, a specific numerical relationship found valid at one time and place cannot be extrapolated to another time and place (Campbell and Sloan 1978a,b, Campbell 1976). The overall concept of pest management system development

presented by Campbell (1973) is structured at least in part with this specific problem in mind. Moreover, there is no established practice of using computerized models as planning aids for resource planning in the eastern hardwood forests infested or threatened in the near future by the gypsy moth.

The prospects for modeling for the gypsy moth analogous to that for the spruce budworm, the Douglas-fir tussock moth, and the Dendroctonus species (mountain, western, and possibly southern pine beetles) are not very favorable. The complex pattern of forest land ownership, widely varying and sometimes unformulated management objectives, and mixed pest management responsibilities seem to require much more elaborate planning and assignments of responsibility than those contemplated for general use in agroecosystems (Glass 1975); education of the grower, with some consultant support, might well prove adequate for agroecosystems. The economics of forest resource utilization in the Northeast may be incompatible with the degree of intensive surveillance of biological and meteorological factors suggested for the cereal leaf beetle (Haynes and Tummala 1976, Campbell and Sloan 1978b).

This discussion summarized the proposed overall viewpoint of the problem of improving operational systems performance. The major thesis is that systems work should not be restricted to numerical modeling or statistical analysis but should include the full scope of all activities important to the effective operation of pest management.

The Development of Ideal and Feasible Systems

Experience has shown that it is desirable to consider several system planning and design targets. The targets should include both short-range and long-range possibilities, and should also include ideal systems, unhampered by real-world constraints, together with some compromises needed to make the ideal systems feasible in the real world. Some of the targets should be systems that would be feasible if some of the more severe real-world constraints were relaxed. For the gypsy moth pest management system, the constraints include predictive capabilities, agents (chemicals, biologicals, parasites, etc.), regulations, public preferences, and agency responsibilities, among others. Time, planning, and effort are needed to obtain any relaxation of such constraints, so the targets are future systems. The system initially implemented should include subsystems intended to achieve some relaxation of constraints and to implement phased improvements which could result from the relaxation. Therefore, the design process should start with a consideration of what future gypsy moth pest management systems might be like and what would be required to make them operationally feasible. It should be understood that anything said in the following pages about some future gypsy moth pest management system is conjectural and draws upon the general body of systems theory (for example, Mesarovic and Takahara 1975).

The ultimate, ideal goal of any comprehensive gypsy moth pest managment system is to work itself out of a job. The theoretical avenues to this goal are to: Eradicate the insect, eradicate all susceptible host plants, or bring about reliable natural control to innocuous, or noneconomic, levels. The first seems to be technically infeasible, and the second is at best a very long-range and remote possibility that depends upon genetic improvement of susceptible tree species. Program research has not pointed the way to reliable natural control. The plan for a comprehensive system must nonetheless include provisions for working toward the system targets while maintaining the best control achievable over the pest population.

At this time, the only known, reliable way to manage gypsy moth populations is to use pesticides; this is understood to include biologicals as well as chemicals, if the conditions are suitable. Near-term plans must therefore be structured around the use of pesticides. Any short-range improvement in pest management system performance ultimately must depend upon using these materials in a more discriminating and cost-effective way. If this is possible, it depends upon improvements in the information available to pest managers and in the way the information is used to plan actual operations.

Long-term possibilities are less constrained, but they depend upon results of ongoing and future research, including foreign exploration for suitable natural enemies. Five major areas can be specified as particularly important.

Continued Search for Effective Natural Enemies

There seems to be no very good reason to believe that there is some still-unrecognized hymenopterous or dipterous parasitoid that will solve the gypsy moth problem. Although the possibility exists, it becomes steadily more remote as more candidates are tried. There are two modifications to foreign exploration that can be attempted. The more important one is to place much heavier emphasis upon disease organisms. It is recognized that importation could present difficulties, but that is still the area with the greatest potential. The second is to promulgate manipulative experiments in areas where the gypsy moth is rarely a problem, to identify the causes of mortality that lead to effective natural regulation.

Identification and Quantification of Mechanisms Leading to Outbreaks in North America

Several theories concerning the mechanisms of release have been proposed and are discussed in chapter 4. According to these theories, several major factors may be involved in the initiation of an outbreak. The theories have not reached a state of refinement adequate to allow quantitative forecasting of magnitudes of population changes. For example, weather is proposed as an important factor, but it is not clear at this point what the precise effects of weather are, much less what, if anything, might be done in a practical system to counter weather effects favoring gypsy moth outbreaks. Another example can be outlined as follows. It is certainly true that a very large outbreak can develop from a small initial focus. However, in a region where the gypsy moth is already widely distributed, it is not clear to what extent the occurence of a regional outbreak depends upon dispersal from outbreak foci. Such foci are favorable habitats, and they may simply provide sensitive indicators of some regional condition favoring gypsy moth populations. In particular, it is not clear what effect early suppression of increasing populations in outbreak foci might have.

Identification and Quantification of Factors Leading to High Tree Mortality Subsequent to Heavy Defoliation

The best current theory is that tree mortality actually results from the effects of secondary attackers, specifically the twolined chestnut borer, *Agrilis bilineatus* and root rot, *Armillaria mellea*. Defoliation is believed to make the tree far more vulnerable to secondary attacks. It is not clear how this may be related to the extreme variability observed in tree mortality. The risk of very high tree mortality can have a major effect on benefit/cost ratios and therefore upon the relative priorities of possible treatment blocks. Improved forecasts would have an immediate effect upon operations planning and upon the overall performance of the system, as measured by cost-effectiveness criteria.

Identification and Quantification of Factors Influencing Defoliation by Gypsy Moths

Field data show that there is a high degree of variability in the defoliation of stands with the same or similar egg-mass densities. Egg-mass densities as low as 1,000 per hectare may produce heavy defoliation, while egg-mass densities exceeding 5,000 per hectare may lead to less than 50 percent defoliation, even of white oaks. This variability is not adequately explained at this time. The available data suggest that oak-pine stands on sand flats may be much more susceptible to defoliation than other stands. Data from Pennsylvania present a mixed and confusing picture not in complete agreement with this provisional distinction. For many stands, the relationship between defoliation and population density is a major part of the basis for treatment threshold determination. Better resolution of the causes of variation in this relationship is essential.

Development of Data Needed to Simulate the Life System of the Gypsy Moth and its Response to Control Measures

Development of a simulation requires a breakdown of processes into elementary changes and interactions and the reconstruction of each process from these elements. The advantage of the simulation, if it can be done properly, is that it can be used to obtain estimates of the gross effects of conditions or perturbations before or even without specially designed field or laboratory experiments. The interesting responses (or absences of responses) suggested by the simulation can then be investigated in appropriate experiments. The major use of simulation is for ongoing improvement of the system and particularly for the screening of possible integrated control techniques.

Sound evaluations of the performance of possible future gypsy moth pest management systems cannot be prepared at this time, although general systems theory can provide some qualitative guidelines which agree satisfactorily with guidelines inferred by analogy from pest management experience. The agreement supports the idea that the full range of information about management and control systems will benefit pest management systems in particular.

The general guidelines are roughly as follows. An ideal control system holds the system being controlled in an optimal state. According to Ashby's principle of sufficient variety, the control system must have just as many ways of responding as the system has for moving away from the optimal state. If the set of responses available to the control system is too small, then one should expect at best oscillations around the optimal state, and at worst catastrophic instability (Ashby 1960). The system designer's problem is that enough variety can rarely be built in to achieve the ideal, but a comprehensive pest management plan must include provisions for resolving such problems.

The conceptual approach is to outline an ideal system and then to fill in details and realistic complications until a practical, operational system is obtained. The comprehensive system includes activities that direct growth and development toward the current ideal target; it also includes activities that may redefine the ideal target.

A Comprehensive Gypsy Moth Pest Management System

The planning and design of the gypsy moth pest management system are being carried out by a Gyspy Moth Planning Task Force (GMPTF). The members include State and Federal officials with operational pest management responsibilities, researchers and research administrators, and industry representatives. GMPTF is formulating a comprehensive target system for gypsy moth pest management and is developing plans to obtain all necessary approvals and to implement the target system. The activities of GMPTF are being coordinated and assisted by a team of planning and design specialists from the University of Wisconsin. To date, the design work is incomplete, and the eventual form of the target system is still uncertain in some respects. The following outlines the form as currently conceived.

System Elements

Six major system elements have been identified:

- Constant factors.
- Line activities.
- Administration, management, and policy.
- Resources.
- Planning and design.
- Research and development.

Precise and complete characterizations of these are still being worked out in detail. Approximate characterizations are as follows.

- Constant factors are those which are either completely stable in their properties or else change slowly relative to typical system planning horizons (up to 10 to 15 years). They are considered to include the gypsy moth, the forest and associated use patterns, the concurrent pest complex, and public and political pressures.
- Line activities are those following a typical annual sequence, more or less closely tied to measures taken to cope with operational gypsy moth problems.

Line activities are broken down into the following nine components, under the current system concept:

Comprehensive gypsy moth program proposal, which is overall strategic planning and coordination, annually updated with a rolling horizon of 5 to 10 years.

Operations planning, which is detailed annual action planning for the execution and required support of all field activities, specifically including at least the first four of the following:

Pest surveillance, which is annual determination of the distribution, abundance, and biological quality of gypsy moth populations.

Environmental considerations (update), which is annual consolidation and capture of new data concerning interaction of the gypsy moth and gypsy moth control measures with rest of the environment.

Intervention, which is the set of annual actions undertaken to affect gypsy moth population densities or population trends.

Public communications, which is facilitation of information flow and informed public participation, especially in planning and evaluation.

Evaluation, which is the general technical support, especially of planning, including assessment and interpretation of prior experience from all sources.

Information storage and sharing, which is the management (capture, storage, maintenance, retrieval) of technical data, but excluding technical literature.

Training, which is the establishment of new skills.

• Administration, management, and policy covers the usual chores of keeping the whole system working in appropriate directions and in harmony with other related systems.

• Resources, actually resource acquisition, are the mechanisms whereby funding of the system is maintained at levels consistent with the results required of the system.

• Planning and design provide continuing system improvement and, it is hoped, progress toward the ultimate ideal goal of reliable natural regulation with no human involvement.

• Research and development produce data, theoretical constructs, and techniques that are needed for continuing system improvement.

The overall pattern of system activities is driven by three major factors: The life cycle of the gypsy moth, the budgeting process, and the pattern of legal and administrative requirements that apply to pest control, most particularly the environmental impact statement. A partial system timeline, shown in figure 8–1, emphasizes the line activities. In most cases, the lines indicate times of peak activity; some may continue at lower levels throughout the year. The connections indicate that the activities are tied together, primarily by information flows. Detailed definitions and systemwide reconciliation of the information flows are currently being worked out by GMPTF.

Overall System Operation

A provisional, very large-scale description of the overall operation of the system is described in the following paragraphs. It must be kept in mind that the planning and systems design work for the comprehensive system is in an early stage; the overall structure is still subject to change. Moreover, the large-scale characterization of the information flow reflects a personal opinion of the most important features at this stage of development, and may differ significantly from what finally emerges from the work of GMPTF.

Comprehensive Gyspy Moth Program Proposal

The purpose of this proposal is to provide higher level, long-range planning and strategic guidance and coordination for the conduct of other activities, especially the detailed annual planning and execution of field operations. In a business corporation, this would be done by the board of directors and upper echelons of corporate management; however, gypsy moth pest management responsibilities reside in a collection of State and Federal agencies, and no pyramidal organization of authority exists. The planning horizon should extend over several years and probably should be adapted to the activity considered. For example, a 5-year planning horizon is too short for gypsy moth research and development strategies because some large-scale natural system processes do not progress that rapidly. Similarly, a

long planning horizon is needed for aspects involving silviculture and stand management. On the other hand, a 5-year planning horizon may be longer than necessary for outbreak suppression operations in the generally infested region. CGMPP concerns itself with such aspects as total system funding and higher level allocations to major subsystems; assignment and

coordination of responsibilities, especially interagency; proposed objectives for other system activities (for example, large-scale suppression of outbreaks to retard spread); and criteria for use in detailed operations planning (for example, weighting of risk of mortality of nontarget organisms in suppression operations planning). The activity would be carried

System elements	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Constant factors	Environment														
						Defoliation		Gypsy moth							
	Concurrent pests														
	Public, political forces														
Line activities	Pest surveillance														
	Information storage and sharing									Evaluation					
	Comprehensive gypsy moth program proposal				Environmental considerations (update)									
	Operations planning													
			Intervention												
	Training														
	Public communications														
Administration management and policy															
Resources															
Planning and design															
Research and development															

Figure 8-1.—*CGMPMS timeline.*

out by a permanently constituted working group that would include responsible State and Federal officials and representatives of concerned groups such as forest industries and environmentalists. Some permanent administrative structure is needed but has not yet been specified.

Operational Planning

This activity produces the detailed plans for action for intervention, surveillance, public communications, and surveys to collect environmental information needed for future CGMPP strategic planning and detailed operations planning. It will necessarily include detailed allocation of resources and specific intra-agency assignments of responsibilities, complete specifications of tasks for cooperative agreements and contracts, and establishment of schedules for execution of all defined tasks. Operational planning should extend up to the point at which particular local conditions must be taken into account in the final action decision. The environmental impact statement will be produced as part of this activity.

Intervention

The actions intended to manage gypsy moth populations are classified as intervention, which includes exercise of management control and administration of field activities and of contracts. It is essentially implementation of the operational planning, supplemented by any necessary on-the-spot decisions. It consists of both field activities and management and administration of field activities.

Surveillance

This action produces the gypsy moth population data needed for the planning activities. These data apply to detection and delimitation of outlying infestations as well as to the planning of treatment programs in the generally infested area. The surveillance activities should also produce data for posttreatment evaluations. The concept of surveillance as specified by GPMTF restricted it to information concerning the gypsy moth populations. The activities are primarily field activities, and the management and administration of field activities, or of cooperative agreements and contracts for them. Field decisions needed to adapt planning of surveillance to onsite conditions are included in the surveillance function.

Environmental Considerations

As a separate line item, this involves several kinds of activity. They include as one major element field surveys and observations of interaction of gypsy moths and of control measures with the rest of the environment. According to provisional GMPTF definitions, they should include acquisition of stand data or tree data for treatment decisions and some of the data needed for evaluation, such as foliage protection achieved. Environmental considerations will sometimes involve collection of data from stands for establishing susceptibility (the degree to which the stand is a favorable habitat for gypsy moths) and vulnerability (the extent of tree mortality, if a defoliating outbreak does occur); the locating of files of data already captured and the making of arrangements for duplication of or access to such files, to improve evaluation and planning; and interpretation of data. Initial creation of the data base and of the subsystem for managing it will be a major task. Subsequent updating and refinement are expected to be relatively easy.

Public Communication

This has two major interrelated aspects. The first is a recognized public information function, the general purpose of which is to facilitate the flow of information to the public and to related systems (legislative, budgeting, higher level administrative, etc.) concerning CGMPP, immediate operations planning, goals and objectives, and longer range plans and research, and general information about the gypsy moth and the effects and economic impacts of the gypsy moth. The public information activity implements and adapts as necessary the plan for the information flow developed as a part of the operations planning. The second major aspect

provides for a reverse flow of information, especially from the general public and from members of particular groups affected by the gypsy moth or by pest management activities. It is particularly important for the reverse flow to include information concerning system performance evaluation criteria for two reasons. The evaluation criteria are used in planning to arrive at treatment decisions, and they are used to help evaluate the quality of the results obtained and thereby identify and assign priorities to possible improvements in the system. The structuring of information flow in the two directions must be intimately coordinated to insure that the planning data concerning criteria are valid.

Evaluation

These activities include as their most important elements the comparative evaluations needed in the overall policy formulations and updates and in operations planning, as well as in the evaluations for the four main action items: Surveillance, intervention, collection of data for environmental considerations, and public communications.

Some evaluation, often with limited objectives, is involved in all other systems activities. Overall evaluation of prior results and comparisons of proposed courses of action requires more than a simple consolidation of the results of such evaluations of limited scope. One major reason is that comprehensive system goals are determined partly by biological factors and partly by socioeconomic factors, and these factors are only partially correlated. In particular, the value of biological information in a total system context will not be the same as its value in a local operations planning context. For example, there could be differences in stand data needed for strictly localized decisions that would prevent useful cross-comparisons. The value of the data would then be seriously compromised in the total system context. Comprehensive evaluation is an essential part of coordination.

Information Storage and Sharing

These activities constitute the data base management for the total system and provide the data needed for planning at both the program proposal and detailed operations levels as well as for various evaluations. Major activities include the capture and editing of data recorded in the field and the update of files; data base acquisition, exchange, or sharing; and maintenance of programs (or procedures, for manual portions) for manipulation and analysis of the data. Provisionally, it is assumed that data peculiar to gypsy moth pest management are handled by the system internally. Data bases of general interest may be duplicated internally or may reside elsewhere and simply be accessed by the gypsy moth information storage and sharing subsystem, depending upon the relative costs and efficiency.

Training

Finally, among the line activites, training has two major functions: It provides new personnel with basic skills in the techniques used in system operations, and it provides all appropriate personnel with basic skills in new techniques and procedures being made operational.

Flow of Activities

The main flow of line activity is strategic planning, detailed operations planning, implementation, data capture, and annual program evaluation. Training is normally concurrent with detailed operations planning; in some cases, at least implementation of training plans will probably be offset by a year. The main input to each line activity from other parts of the system is information. To a substantial degree, lists of data items of potential interest have been compiled from research findings and from less structured field observations. The data items include both biological and ecological factors, which form the basis of forecasting and also include socioeconomic elements. The relative values of the proposed items and possible requirements for additional items are not completely clear at the time of writing; in fact, the collection, editing, organization, and analysis of data are just now approaching the point at which some crucial large-scale cross-comparisons can be made.

The timing of these cross-comparisons depends upon at least two uncontrollable complexes of factors,

the natural process leading to an outbreak and the process affecting the trees subsequent to an outbreak. Some of the critical data will not be available until observations from the 1978 field season have been captured and edited. Other data elements will not be available even then, because they depend upon analysis of similarities or differences among different outbreaks in the same stands, and these have not occurred.

The inability to carry out some of the crucial cross-comparisons emphasizes the importance of some of the activities listed as other than line activities, especially research and development, and planning and design. The scope of the available data will almost surely not be found adequate to explain differences among outbreaks in different places. The system as conceived will identify and document the existence of differences and provide some data, normally collected in conjunction with the line activities. This will naturally lead to requirements both for research and development and for planning and design activities. The interactions are again primarily exchanges of information within the system; the extent to which internal resource flows in terms of manpower, physical resources, and funding can occur is not resolved at this time. The characteristics of the information flows needed are relatively clear, however. Identification and documentation of problems come about through the evaluation activities, drawing upon the information storage and sharing function for the data. Resolution can be expected to involve research and development activity. The involvement could be only at an analytical and interpretive level, based upon the stored data, but normally would require some new research. Incorporation of solutions back into operations of the line activities involves planning and design as an intermediate step. Evaluation of possible operations is also involved, in obvious ways, in the planning and design.

Some overall system administration, management, and coordination are essential. From an organizational and administrative point of view, fine-scale operations are ultimately under local control. This implies a need throughout the system for heavy emphasis upon coordination, maintenance of general

public support, and maintenance of funding and possibly other resources (for example, manpower resources and dollar resources can become very difficult to reconcile).

The Federal role in gypsy moth pest management will be significantly influenced by these systemwide problems, but they are not resolvable entirely at the Federal level.

The GMPTF has favored the formation of committees and working groups, or the use of some already in existence, for much of the planning and evaluation activity. In view of the diffuse, multi-agency, and seasonal nature of the problem, this seems to be a practical approach. There is also a need, recognized by GMPTF, for some permanent, continuing activity. Some of the necessary year-round continuity could be provided through a Federal agency, and there are other workable alternatives. The organizational question, which has not been decided yet, promises to be difficult to resolve.

Modeling and Systems Development

The desired output of the systems work within the gypsy moth program is implementation of any possibilities for improvement of existing systems for coping with the gypsy moth.

Watt (1961) published an extended discussion of the general problem of developing mathematical models for insect pest management. He views the problem as a relatively long-term research and development project, with a suggested planning horizon of around 20 years. He discusses, at least at the conceptual level, the planning of field work, data handling and analysis, development of functional (or process) models, and possible uses of mathematical optimization techniques. Watt provides enough detail in this and in a subsequent paper (1963) to give reasonable assurance that the kind of project he proposes is technically feasible, given an adequate duration.

In view of the widespread conception of systems work to be primarily the development of numerical predictive models of various kinds, some discussion of the place of modeling and of some elementary distinctions among kinds of models seems necessary.

The distinctions to be drawn are not rigorous and absolute, but are often matters of shifting emphasis, and the boundaries are not sharp.

Figure 8-2 shows a simple outline of the relationships between models and real systems, as they are considered here, and also indicates some of the major uses of models. The three kinds of models considered here are referred to in this chapter as conceptual, statistical, and functional.

A conceptual model of a system is a predominantly verbal or diagrammatic description; a naturalistic description of the life system of the gypsy moth is a conceptual model. Other examples include organization charts, structural formulas of chemistry (such as

CH_4 for methane), flow diagrams, blueprints, and architect's renderings. They are essential in formulating a conceptual design for a humanly purposeful system, and the conceptual design is itself a conceptual model of the proposed system. In many cases, successful implementation may proceed easily and directly from a largely conceptual model.

Statistical models are basically quantitative and are most commonly used to attempt to describe relatively gross or large-scale relationships between separate features of some entity in a simple way, without involving many structural or functional assumptions. In this sense, they are intended to provide a compact, economical statement of gross relationships that are
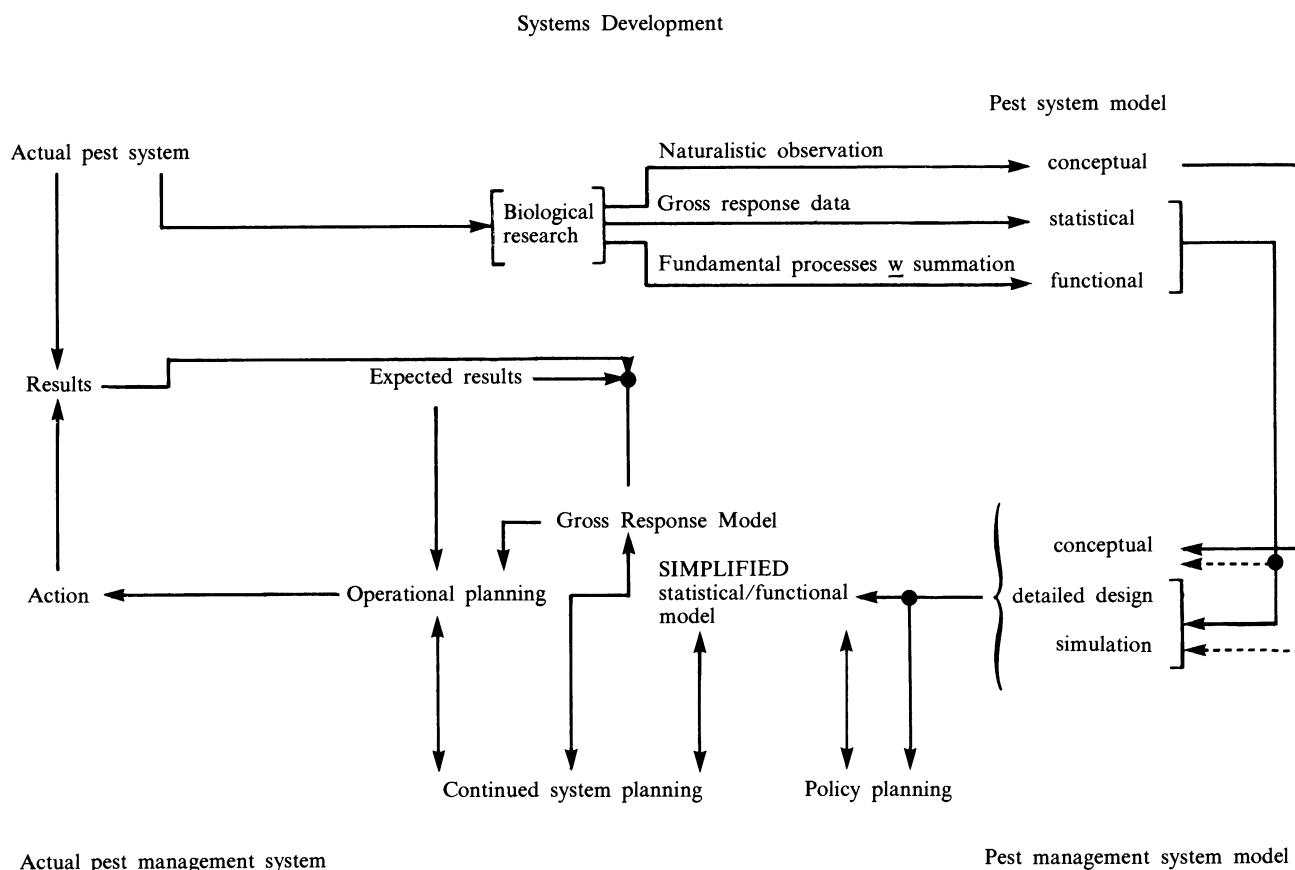
Figure 8-2.-*Outline of relationships between real systems and models.*

present within some particular set of data, and they emphasize provision of estimates of risks of associated errors or estimates of tolerances.

Functional models are based on assumptions concerning finely detailed, elementary interactions within some process, and use summation or integration techniques to attempt to predict or explain, in terms of elementary interactions, gross relationships or system responses. Such models are most commonly used in physics and engineering. Most simulation models are functional models. They tend to be complex and difficult to use, and are most often used in systems development to form preliminary estimates of the possible importance of various system features, with the unltimate goal of improving the efficiency of the system. Functional models are most obviously useful and dependable for systems deliberately constructed in a specific, well-defined way from components whose behavior and interactions are known. They can usually be developed from conceptual models of deliberately constructed systems and form the basis for quantitative analysis before the system is actually constructed. In these cases, a variety of system models is normally developed and used to explore particular aspects of potential system characteristics, such as the effect of some particular component on system performance and cost. They may be used in investigations of incompletely known systems to test assumptions, formulate problems for research, and, after considerable validating experience, to formulate predictions of system behavior in untried situations. Complex functional models are of most use in research and development activities. They are sometimes necessary for long-range operational planning; they are often too slow, expensive, and unwieldy to be very satisfactory for short-range planning.

One common method used to obtain forecasting equations useful for real-time projections is to approximate the output of a complex functional model by means of general data-fitting techniques. Normally the fitting can be done in such a way that the output of the model is approximated by some simple function of input variables corresponding to

quantities observable through reasonable sampling procedures. The result is then compatible with the use of well-standardized statistical procedures.

A large-scale version of a diagrammatic pest system model is shown on figure 8–3, which shows major functional areas of a comprehensive system, as conceived by Ketron, Inc., in a preliminary systems design study (Blacksten et al. 1977). It shows major functional areas involving suppression, considered to take place in the generally infested area of the Northeast United States; long-term control; naturalization, producing good regulation by natural enemies if this is feasible; containment, which lies within the APHIS charter and is intended to limit the size of the generally infested area; and an overall systems support function, needed to maintain and improve systematically the performance of the major pest management system elements. A possible breakdown of functions within these major areas is also indicated.

Figure 8–4 presents a timeflow chart for certain major activities carried out to accomplish the suppression function. Most of the activities would have close analogs in a timeflow chart for accomplishing the containment function. Naturalization is substantially different and appears to be highly uncertain and problematical for a gypsy moth pest management system. Figure 8–4 also indicates where in the pattern of activities the use of suitable quantitative models would occur. Since the flow describes certain line operations, the models used in the scheme can be regarded as primarily statistical in character. The models are intended to forecast major responses using sampling data for current-season decisions, without involving fine details of the processes, and they provide error estimates highly desirable for the decisionmaking process.

The purpose here is to illustrate the interplay of modeling, systems development, and system operations. It should suffice to select defoliation forecasting as an example problem for discussion, because this is an element of critical importance in damage forecasting. Campbell and Sloan (1977) point out some of the problems associated with the different susceptibilities of trees to defoliation and suggest use of a derived defoliation potential, referred to white

oak (*Quercus alba*), as a base. The exploratory studies of data from the intensive plot system (IPS) suggested as a complementary method that the forecasting might start with a forecast of white oak group defoliation (essentially white oak, *Q. alba*, and chestnut oak, *Q. prinus*). The extension to other species categories is, as Campbell and Sloan point out, not altogether simple, and a completely satisfactory solution has not been found at this time for use in an overall defoliation forecasting scheme. The problem is particularly troublesome in case the white oak group defoliation exceeds around 95 percent.

Figure 8–5 shows a plot of predicted site defoliation against observed site defoliation, using a regression equation proposed by Campbell and Standaert (1973). The comparison is *not* a satisfactory test of their defoliation equation. In all cases shown, the gypsy moth populations were collapsing; the
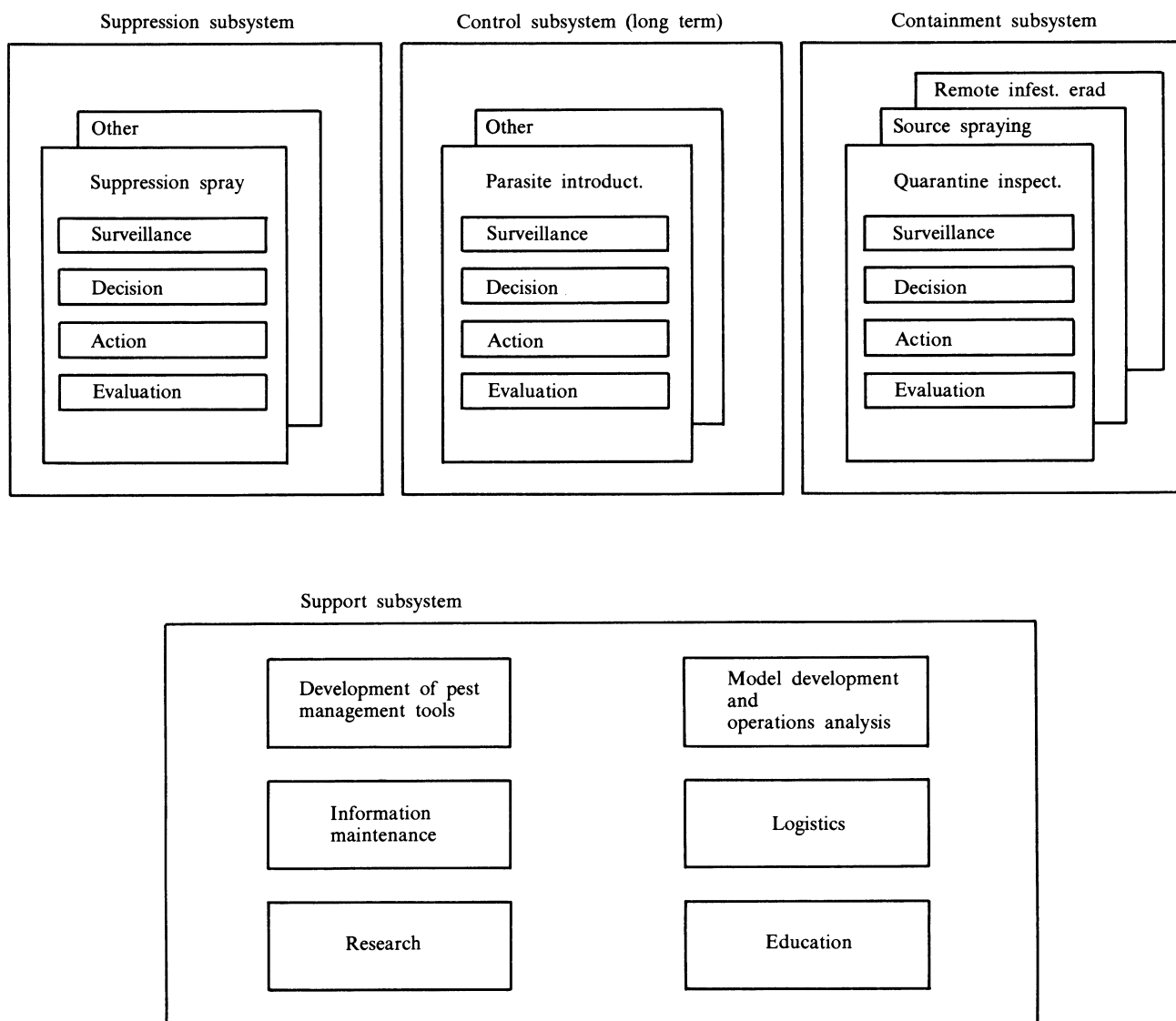
Figure 8–3.—*Gypsy moth pest management system.*

Key:

| Activity making use of program-developed models |

User activities

Data

N — Criteria Met? — Y

SYSTEM startup

1
User establishes work units

2
Devise cost-effective sampling plan for population monitoring

{ Annual cycle

3
Carry out phase one sampling

4
More sampling needed? — N

5
Conduct phase two sampling

6
Predict defoliation levels

7
Project defoliation impact, tree condition and refoliation

8
Project tree mortality rates and volumes

9
Project growth volume loss

Stand data from 3 or 5

10
Compute and discount value of projected growth and mortality

11
Add special costs to get total cost of not treating

12
Evaluate treatments in terms of net benefits

Information on effects, reliability, and cost of treatment

13
Schedule units for treatment in order of benefit/cost ratios

No treatment

14
Perform pretreatment evaluation and last-minute population estimate adjustment sampling

15
Update population projections

Treat — N

16
Treat and take evaluation sample

17
Critique equipment, field operations and personnel, and report on problems found

18
Evaluate biological responses for "outliers" and report results to proper unit

19
Evaluate system predictions for evidence of trend

20
Report results of evaluations to proper unit

21
Update sampling resource allocation methods and information on reliability of trial

Next cycle

Figure 8–4.—*Time flow chart of a pest management system.*

Figure 8-5.—*Predicted* vs. *observed defoliation.*

importance of this aspect is unclear. The main drawback to Campbell and Standaert's equation is that the defoliation forecast for year y requires densities of egg masses deposited in year y-1 and in year y-2. The monitoring of gypsy moth populations, under current system plans, will not necessarily provide data concerning egg masses deposited in year y-2, although it will provide data concerning ·egg masses deposited in year y-1. It is anticipated that, in the near future at least, defoliation maps will be used to select places where egg-mass counts will be performed. Some counting will be carried out in areas not showing much defoliation, but there is no assurance that data from prior years will be available.

Figure 8–6 shows the relationship suggested as a result of the exploratory analyses of the intensive plot system data, for white oak group defoliation. The solid line represents the provisional forecast; some of the data points are also shown. Other points have egg densities outside the plotted ranges, especially for the sand flats, and defoliation of at least 99 percent.

Benefit/cost analyses to support treatment decisions are discussed in some detail in chapter 7. As is pointed out, the details of an analysis must be adapted to the immedidate situation. Cost components for the homeowner in a forested community, the owner of campgrounds, and the owner or manager of commercial forest stands are all considerably different. For present purposes, we observe that tree mortality is in all cases a potentially important element; the extent of tree mortality subsequent to a severe outbreak is notoriously variable. Chapter 5 contains an extensive discussion of the effects of defoliation on trees, including mortality. At this time, forecasts of the extent of tree mortality subsequent to severe defoliation are still problematical. Preliminary analyses of overall tree mortality data from the intensive plot system indicate that the tree (or crown) condition at the beginning of the outbreak was particularly important. The condition assessment used was based on visual examination, taking into account such factors as crown density, presence of dead limbs and branches, and epicormic branching. Closer examination, not yet complete, has not revealed any clear relationship between observed mortality rates of trees in poor condition and prior defoliation. This apparently differs from the data from the Melrose plots (Campbell and Valentine 1972).

Table 8–1 shows an overall summary of the intensive plot system experience for oaks. It should be noted that most trees that experienced high defoliation in 1972 also experienced it in 1973. The mortality rates shown are not intended as estimates or planning allowances for operational planning; further analysis is needed for such purposes. The tabulated rates are intended only to indicate what was observed over a variety of stands and are used here for illustrative purposes only. Tree values vary enormously, depending upon the use of the tree. If tree mortality at the rates shown occurred in a commercial forest managed for sawtimber production, the loss per hectare could average over $250. Since treatment costs using pesticides currently average less than $25 per hectare, benefit/cost ratios would strongly favor treatment. This would remain true even at
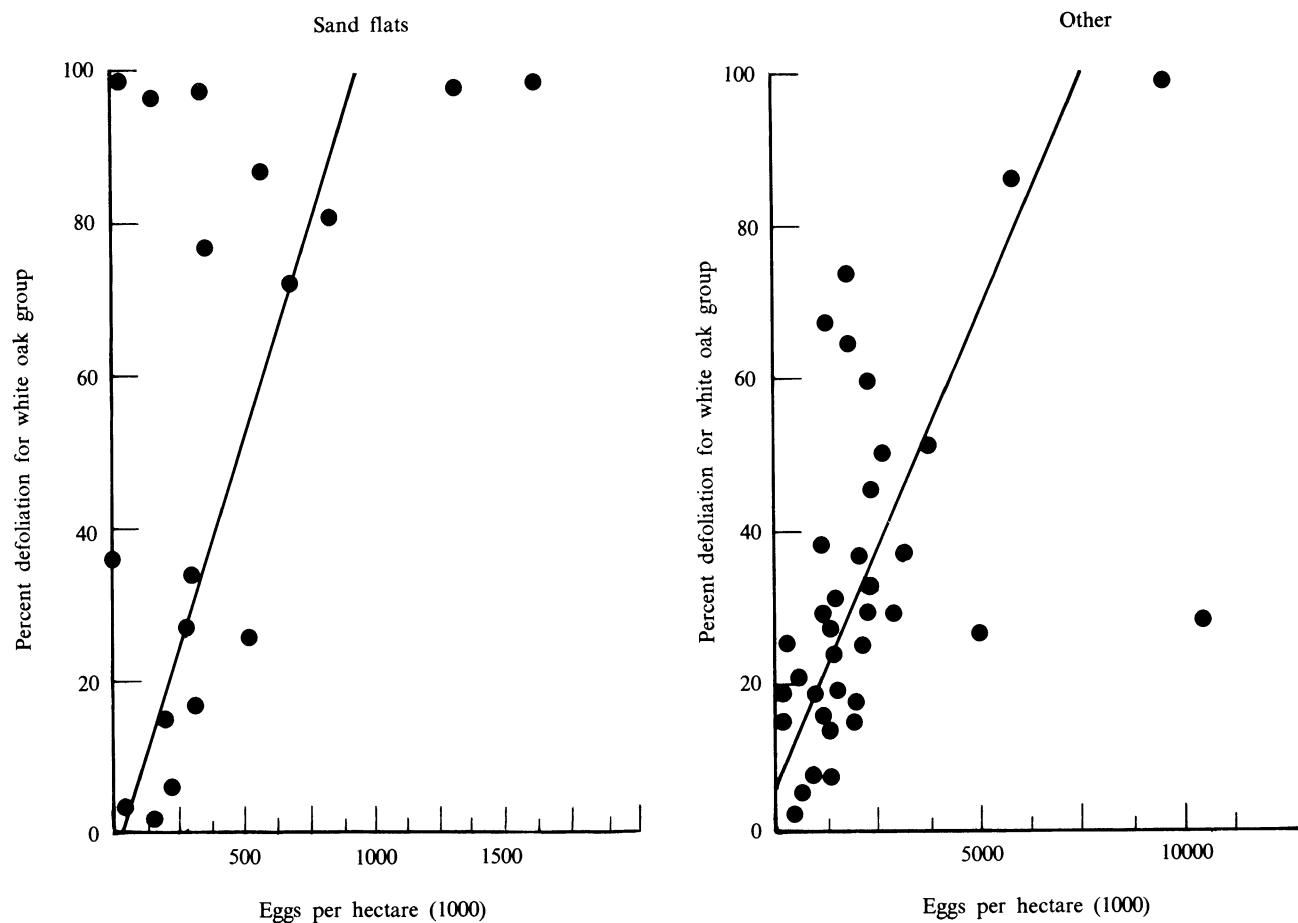
Figure 8-6.—*Provisional relationship of white oak group defoliation.*

Table 8-1.—*Example of tree mortality experience*

1972 Percent Defoliation	1972 tree condition								
	Good			Fair			Poor		
	0–40	40–90	90–100	0–40	40–90	90–100	0–40	40–90	90–100
White oak group:									
Number of trees	401	114	150	133	79	312	52	24	144
Percent dead in 1976	1	4	21	5	8	25	56	62	49
Red oak group:									
Number of trees	514	232	510	314	79	201	71	25	31
Percent dead in 1976	4	3	3	9	19	11	39	52	48

substantially lower tree mortality rates, with the assumed economic factors: 125 to 250 white oaks per hectare with a stumpage value around $10 per tree. Residential and campground tree mortality would usually lead to losses several times as large. Stands in which tree values were considerably lower would require analysis adapted to the specific conditions and management objectives.

Optimization Considerations

The use of an economic threshold for pesticide applications has been specified as one of the characteristics of the more sophisticated formulations of pest management tactics (Van den Bosch et al. 1971). Headley has pointed out that this approach, which he describes as "treat as needed," might benefit from some refinement (Headley 1975). Mann (1971) has published a theoretical study of the use of dynamic programming (Bellman 1957) in the formulation of pest management tactics, and Shoemaker (1976) has discussed the possible uses of optimization techniques. Watt (1963) discussed the possible use of dynamic programming for forest pest management in particular. An application of decision analysis (Howard 1971, Raiffa 1968, Raiffa and Schlaiffer 1964) to gypsy moth suppression is discussed by Talerico et al. (1978). Some basic implications of the possible use of optimization techniques for the case of the gypsy moth are described below.

A simple model of particular interest can be defined as follows. Assume that the effect of a treatment is to kill a fraction of the pest population, this fraction being independent of the population density but dependent upon the intensity of the treatment (for example, the dose rate). Assume that the population density in the subsequent generation is representable as the product of an independent random factor and the density of survivors of the treatment, with the random factors forming a set of independent, identically distributed random variables. This leads to a product form for the population dynamics equation, which can be transformed into an additive form by a logarithmic transform. The necessary algebra is found

in Historical Review, in chapter 4. The formal problem is to optimize the pesticide treatment.

Discussions of the use of pesticides in pest management emphasize the need to select some treatment threshold, often called an economic threshold or economic injury level. The economic threshold, at least by implication, should be derived with the aid of some optimization method. The form of the tactic is to apply a standard, predetermined dose if the pest population exceeds this threshold. The only dimension considered for optimization is pest population density. This places a significant constraint on the problem. The range of possible solutions can be extended by removing the constraint on the dose rate, thus allowing decreased or increased rates to be considered. The following discussion concerns this expanded problem.

Specifically, it is assumed that the dose rate can be adjusted to minimize the sum of treatment costs and resulting costs of damage caused by the pest. The dose rate selection is therefore allowed to depend upon the pest population density. The cost of producing a particular kill rate is assumed to vary in a regular way with the kill rate. The kill rate is assumed to be determined by the dose rate. Theoretically, it does not matter whether the dose rate is controlled by adjusting the concentration of active ingredient in the spray formulation or by adjusting the application rate of the formulation, with the concentration held constant, or by some intermediate procedure. For convenience of discussion here, it is assumed that the application rate is controlled. Note that this means that the application rate is varied as necessary to produce an optimal kill rate, and not that the rate is held accurately fixed.

The dynamics of such a model can be made formally analogous to production and inventory control models, with the exception that certain positivity constraints generally imposed on probability distributions for demand in inventory control models are not necessarily satisfied in the pest control model. Many results of inventory control theory therefore apply to the pest control model. The results of particular interest here concern the mathematical form of optimal policies; this form depends upon the form of the cost relationships. For present purposes,

these cost relationships are assumed to have the following particular form. The treatment cost is assumed to be a linear function of the logarithm of the survival probability, and the damage is assumed to be a convex function of the logarithm of the density of survivors. Other forms might also be suitable (Mann 1971). If there are no fixed costs of treatment and if the variable costs of treatment and the damage costs have suitable shapes, then the optimal treatment has a remarkably simple form, such as follows. There is some critical pest population density K such that if the population density exceeds K, then the treatment is selected to reduce the population to the level K; otherwise no treatment is carried out. If there is a fixed cost of treatment, then there is a pair of critical numbers, k and K, with $k > K$, such that no treatment is carried out unless the pest population exceeds k, in which case the treatment is selected to reduce the density to K.

It is noted that the optimal policies for this simple system do tend to produce an approximation of homeostasis, in agreement with the general principle of sufficient variety discussed earlier. The variety in the controlled system arises from the generation-to-generation changes, and the controller acts to cancel undesirable transitions.

It is known that the critical values change if the planning horizon changes. If the pest population is certain to increase, then the critical levels decrease as the planning horizon increases; otherwise, the effect of changing the planning horizon may be irregular. It is also known that if the basic cost functions fail to have the proper shapes, then the optimal treatment selections can depend upon the pest population in very complex ways.

Quantitative estimates of the results of using such tactics can not be made with any assurance at this time for gypsy moth control, because no data base exists for field dose response. However, elementary calculations suggest that both the shape of the dose response curve and the shape of the curve representing the relationship between population density and economic impact are very important; either one might prove dominant. Variable application rate tactics are relatively more advantageous, compared to fixed

application rate tactics, if the slope of the dose response curve is relatively low. Laboratory data suggest that these slopes for chemical pesticides are relatively steep in comparison to the slopes of the impact curves, and this in turn suggests that relatively little might be gained. Field conditions involve distribution, behavior, and competitive mortality factors, however, and some of these could affect dose response curve slopes significantly. Some of these, at least, should tend to reduce the dose response slopes. Biological agents such as Bt and gypsy moth NPV have laboratory dose response curves with lower slopes, and the theoretical advantages of variable application rate strategies could be realistically very significant for such agents, when used in a pesticide mode.

Forest insect pest control practices have never attempted to match pesticide application rates to pest populations. The reasons for this neglect are not altogether clear. Several possible disadvantages are easily identified, however. The most obvious is that there is very little field dose response data that could be used to select application rates. Some problems in controlling application rates to the desired values would also be encountered, especially for aerial spraying using fixed-wing aircraft. There could also be some administrative and legal problems associated with treatment efficacy concerning pesticide registration, labeling, and use. Finally, variable application rate policies would require more accurate and detailed pest population data than would fixed-rate policies. The direct benefit in reduced chemical pesticide cost would not be very great, so there is not much direct economic incentive to undertake the job of developing and implementing such policies under current conditions. It should be noted that agricultural and orchard pest control economics are very different from gypsy moth pest control economics, and variable application rate policies for them could be of considerable direct economic interest.

Consideration of just the direct cost of the pesticide may be misleading. Criteria for good pest-management practices include the goal of using as little broad-spectrum pesticide as possible, which implies that there is some penalty, or indirect cost, that

should be added to the direct cost of the pesticide. There is no universal prescription for quantifying the added penalty, although reasons for claiming it exists have been thoroughly and extensively documented. Accordingly, it would not be prudent to dismiss variable application rate policies for possible use in gypsy moth pest management. The first problem, from the point of view of implementation, would be to determine if variations in application rates produce variations in response that are sufficiently consistent and predictable to be operationally useful. A number of factors are known to be, or suspected of being, involved in the response to pesticide application. Some of these, such as weather and timing, are not subject to control or long-range prediction. Factors related to foliage quality and to pest population quality are suspected of being important, but not enough is known currently to permit quantitative analysis. Development of operationally satisfactory methods for sampling or measuring such factors could require considerable time and effort.

Given that an accurately controlled response could be achieved to variations in application rates, significant improvement in aerial spray technology would still be required. The required improvements are feasible technically, but again considerable time and effort might be required to obtain economical solutions. Intermediate solutions could be obtained by suitable layout of spray blocks with variation of application rates allowed between but not within blocks.

The selection of suitable thresholds or critical levels of optimal treatment rules is, in principle, based on a quantitative benefit/cost analysis. In fact, the formal analyses are most useful for large-scale and long-range planning associated with new system conditions or changes. For highly specific local operational planning, computed thresholds and treatment intensities may not adequately represent the local preferences and priorities. Some adjustment of the critical levels should be allowed so that these can be represented better.

It is not uncommon to find that some application of judgment is needed to establish fine details of general rules for some policy. Some examples from other systems, all involving some application of judgment to the selection of some basic control parameter, may be helpful in providing some perspective.

- Selection of a safety level of supply for a blood bank.
- Selection of an amount of ammunition to carry into combat.
- Selection of a ratio of cash reserves and other liquid assets to long-term capital investment.

In general, analysis can be used to establish initial values for such selections, but experience and judgment are used to adjust such selections as necessary. Some guidelines and often some analysis should be available and should be used to assist in decisions to modify the nominal large-scale values properly.

The discussion of optimization is meant to illustrate several points. The three considered here to be most important are that one, unless the scope of the optimization is constrained sharply, the results can be expected to be complex in form, so that some developmental work would be needed for practical implementation, even of approximations of optimal tactics; two, as a consequence, general theoretical results as well as specific numerical results are needed to provide guidelines for systems planning and design; and three, a good quantitative knowledge of the response of the pest population to intervention is essential.

For gypsy moth populations, field dose response data are missing, even for pesticides, and this precludes numerical computation of optimal tactics. Feedback from operations can provide some important guidance, especially if some supplemental monitoring can be carried out beyond what is currently performed. The feasibility of this will be determined by the Gypsy Moth Planning Task Force, and if it is determined to be practical, an integrated program will be planned and implemented.

Functional Models and Research Requirements

Attempts to develop functional models of the interaction of the gypsy moth with its environment very quickly encounter major areas where basic

information is lacking. Sometimes sensitivity analysis can determine that the uncertainty is not very important. More often, the results are unclear; some possible process may be important or not, depending upon other uncertain elements.

The functional modeling effort discussed in the following paragraph was intended to investigate the following questions:

- What functional form is it reasonable to use to approximate the relationship between initial population density and defoliation?
- Is it plausible that the relative phenologies of insect and foliage development could have a major effect on the defoliation relationship and, by implication, on mortality due to starvation?
- Is it plausible that a massive NPV epizootic might develop from an almost unobservable initial NPV incidence, assuming horizontal transmission depends only on ingestion of contaminated foliage?

The simulation does indicate that phenology could be very important, depending upon the relative times of initiation of hatch and bud burst, the subsequent rates of development, and the shapes of the distributions over time of hatch and bud burst. Unfortunately, very little data have been published on these factors.

Doane (1976) has suggested a fairly simple conceptual model for the development of an NPV epizootic, and this was used to structure a substantial part of the epizootic aspects of the simulation. Discussion of a particular, highly simplified model should help to clarify the nature of the modeling problem. One striking feature of gypsy moth outbreaks is the formation of epizootics, especially of nucleopolyhedrosis virus (NPV) in dense populations. There seems to be a lack of agreement among researchers concerning the underlying mechanism. One theory is that the epizootic arises naturally as a consequence of the presumed natural mode of horizontal (within generation) transmission—ingestion of foliage contaminated by polyhedra released from the disintegrating cadavers of insects killed by NPV—and the population density. Another theory is that a latent mode of infection by NPV exists and is common, perhaps universal, in North American

gypsy moth populations and that some stress in dense populations leads to conversion of the latent infection into a lethal frank expression. Both mechanisms presumably could operate simultaneously. Other factors could also be relevant. Gypsy moth NPV incidence seems to increase as the age, in generations, of an outbreak increases, and NPV mortality is reported to be low in populations that remain sparse for several generations. If there are systematic changes in foliage composition subsequent to a first defoliation, then these might also increase stress. Also, a variety of mechanisms could produce at least an occasional frank NPV infection, independently of any conversion of a latent infection.

A simple epizootic model not incorporating activation of latent infections is discussed below, primarily to illustrate the nature of the problems involved in developing a model of the process. One aspect of considerable interest is the possibility of transovum or transovarial transmission; this could be important to an understanding of the dynamics of a gypsy moth outbreak. A simplified compartment model, as outlined on figure 8-7, can be used for some exploratory analysis of possible behavior of gypsy moth NPV epizootics.

The major overall simplifying assumptions are as follows:

1. Some fraction of the newly hatched larvae are NPV infected.
2. Some infected larvae die from the NPV infection.
3. Infectious polyhedra are released into the environment from larvae that die of NPV infection, and some possibly extremely small fraction of these are deposited on foliage.
4. Healthy larvae become infected through ingestion of contaminated foliage. For present modeling purposes, this is assumed to be the only mechanism, other than the undefined mechanism producing the initial infection. This assumption is made in part to examine the plausibility of such an assumption, through the general behavior of the resulting model.
5. Other mortality factors operate equally against healthy and infected larvae. It must be noted
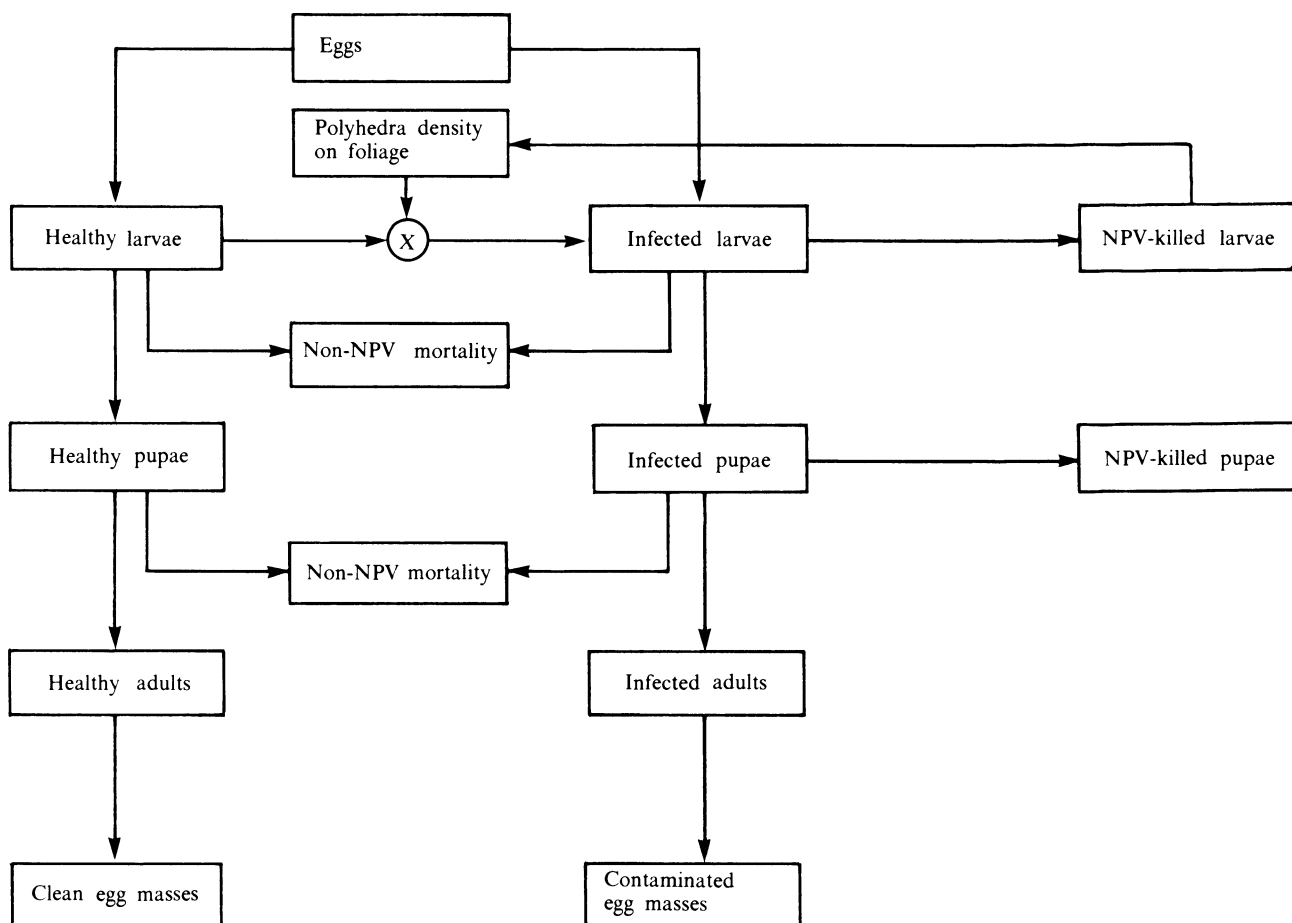
Figure 8-7.—*Simplified gypsy moth NPV epizootic compartment model.*

that this is a simplifying assumption, for modeling convenience only. It may not be valid for real gypsy moth populations.

6. Healthy pupae and adults do not become infected by NPV. This is also a simplifying approximation; for example, parasite or predator attacks could infect some pupae.

7. Infected insects that reach the adult stage produce contaminated egg masses. This assumption is purely hypothetical, intended only to examine quantitative implications across generations.

Two additional major assumptions seem to require some comments and explanation.

8. Contamination of the environment from pupae that die of NPV infections occurs too late to contribute to the development of the epizootic. In fact, examination of pupae in the field suggests that the integuments of pupae that die from NPV infections are not especially fragile. Accordingly, it is assumed that these do not disintegrate or rupture fast enough to contribute significantly to contamination of foliage while feeding larvae are present.

9. The rate at which healthy larvae become infected by NPV is proportional to an instantaneous potential gut load of virus particles, measured in terms of polyhedra and determined from foliage contamination density, foliage ingestion rates, and rates of elimination from the midgut. This assumption is pure conjecture and may be quite incorrect in important respects from a biological process point of view.

During periods of high larval mortality, the density of polyhedra produced and presumably deposited on foliage should change rapidly, possibly leading to a rapid change in effective dose rate. There are only limited data on the time normally involved in passage of material through the midgut; the time is relatively short (perhaps 2 hours) under certain laboratory regimes. Also, feeding behavior of an individual insect under outbreak conditions is not known with any certainty. Allowance for mixing of infectious material in the midgut seems to be the most reasonable approach. For present purposes, the elimination rate is assumed to be determined by the time to pass through the midgut. Possibly it should instead be determined, at least in part, by some rate of destruction of infectious material in or before the midgut. There are no quantitative data to provide guidance.

Table 8-2 lists the variables used in the model, and table 8-3 lists the rates associated with flow between compartments. The system of differential equations making up the functional model is shown in table 8-4.

These equations are not all independent; some of the variables are bookkeeping entries to account for flows. Only 11 equations are needed. The system as written is sufficiently complex for the present discussion. A more complex system has been programmed that incorporates somewhat more of the biology of the gypsy moth, by distinguishing sexes and larval instars. The more complex model has 130 separate compartments. Some of its characteristics will be discussed later. The simplified model can be used to point out research problems that require quantitative answers.

Table 8-2.—*Variables for the epizootic model*

Variable notation	Interpretation
E	Total eggs
L_H	Healthy larvae
L_S	Infected larvae
L_N	NPV-killed larvae
L_R	Non-NPV-killed larvae
P_H	Healthy pupae
P_S	Infected pupae
P_N	NPV-killed pupae
P_R	Non-NPV-killed pupae
A_H	Healthy adults
A_S	Infected adults
M_H	Noncontaminated egg masses
M_S	Contaminated egg masses
V	Polyhedra density on foliage
U	Virus gut load
t	Time

Table 8-3.—*Epizootic model rate parameters*

Rate parameter notation	Value used for example	Interpretation
K_E	0.3	Overall hatch rate (eggs per day)
F_H	.98	Fraction healthy
F_S	.02	Fraction infected
G_L	.05 (t>25)	Pupation rate
	.00 (t<25)	
G_P	.07	Adult emergence rate
R_L	.06	Non-NPV larval mortality rate
R_P	.03	Non-NPV pupal mortality rate
S_L	.20	NPV-caused larval mortality rate
S_P	.20	NPV-caused pupal mortality rate
Q	.0075	Infection rate
C_M	.01	Foliage contamination per larval death
C_V	.5	Decay rate of NPV on foliage
C_F	10.0	Larval feeding rate
C_U	15.0	Midgut evacuation rate

Many of the problems center upon initiation and progress of an infection. To start with, consider the rate parameter Q. It determines the rate at which noninfected larvae become infected. However, the value of the parameter Q is not related in any simple way to traditional dose response data. In fact, most gypsy moth NPV bioassays are not intended to yield

Table 8-4.—*Epizootic model differential equations*

$$dE/dt = -K_E \cdot E$$

$$dL_H/dt = F_H \cdot K_E \cdot E - G_L \cdot L_H - R_L \cdot L_H - Q \cdot U \cdot L_H$$

$$dL_S/dt = F_S \cdot K_E \cdot E - G_L \cdot L_S - R_L \cdot L_S - S_L \cdot L_S + Q \cdot U \cdot L_H$$

$$dL_N/dt = S_L \cdot L_S$$

$$dL_R/dt = R_L \cdot L_S + R_L \cdot L_H$$

$$dP_H/dt = G_L \cdot L_H - G_P \cdot P_H - R_P \cdot P_H$$

$$dP_S/dt = G_L \cdot L_S - G_P \cdot P_S - R_P \cdot P_S - S_P \cdot P_S$$

$$dP_N/dt = S_P \cdot P_S$$

$$dP_R/dt = R_P \cdot P_S + R_P \cdot P_H$$

$$dA_H/dt = G_P \cdot P_H$$

$$dA_S/dt = G_P \cdot P_S$$

$$dM_H/dt = 0.5 \cdot G_P \cdot P_H$$

$$dM_S/dt = 0.5 \cdot G_P \cdot P_S$$

$$dV/dt = C_M \cdot S_L \cdot L_S \cdot C_V \cdot V$$

$$dU/dt = C_F \cdot V - C_U \cdot U$$

absolute dose response information. One technique for obtaining an approximate estimate of Q is to derive a similar set of equations to model a bioassay. This device was used to obtain an approximation for the value, and also to examine the plausibility of the assumed structure. Bioassay data are not very satisfactory, however, since they apply to at least partially purified material and are conducted under laboratory conditions. Even with an estimate of the parameter Q, there are still the parameters C_M (foliage contamination per larval death) and C_V (loss of infectious material from foliage) to estimate. Although there are some data on the number of polyhedra produced, this cannot be translated into an average foliage contamination. There also seem to be no published data on the rate of inactivation or removal of the natural material from foliage. In fact, it appears that there are essentially no published quantitative data concerning deposition, distribution, or persistence of the natural contamination of foliage. For analytical modeling, this means that initial guesses must be made that seem generally plausible; then the parameters must be varied to explore

sensitivity. An objective is then to find combinations of parameter values that seem plausible and that also lead to epizootic patterns generally similar to what is observed in the field.

A close match obviously cannot be expected with so simple a model. The distributions over time do not have the proper shape and the compartments start filling too soon. Accordingly, there are some serious uncertainties concerning particular features. For example, if there is much NPV incidence, then the model indicates that significant numbers of NPV infected adults should be produced, leading to major intensification of the epizootic in the second year of an outbreak. Such fairly good survival of infected insects, as projected by the model, is at least partially a consequence of the very early time at which the adult compartments reach significant levels. The nominal duration of a virus infection in larvae, given a heavy infecting dose of NPV, is about 10 to 14 days, terminated by death; longer durations are observed at relatively low dose rates. It would be important to determine if an NPV infection developed in pupae at the same rate as in larvae with similar gross internal effects. There is some reason to suppose that the infection may follow approximately an exponential growth internally, with rates that may vary among different cell types and tissue structures. If the growth rates of virus in larvae and pupae are similar, then survival to the adult stage of an insect infected as a larva would presumably be unusual. Direct information about the internal course of the disease is very sparse. The theory of branching processes suggests the following two conjectures: Some NPV infections will die out at a very early stage, especially if very few cells are involved, and once the infection involves the hemocytes and a few hundred cells are involved, extinction of the infection should be exceedingly improbable. An extinct infection should leave a few polyhedra some place in the body of the insect, probably in the hemolymph. Conditions in the hemolymph are supposedly inappropriate for lysis of the polyhedral protein matrix, so the presence of such polyhedra should not lead to a continued or renewed infection. Possible consequences of phagocytosis of polyhedra seem obscure. Better information concern-

ing the process of an NPV infection in an individual is necessary.

The model outlined is too crude for very specific conclusions. It does suggest several interesting possibilities, however, and it also calls attention to some interesting questions. The qualitative feature of most interest is that the model does yield highly density-dependent solutions. That is, it indicates that overall NPV mortality is very small if the initial value of E (total eggs) is small and the factor F_S (fraction of newly hatched larvae infected by NPV) is fairly small (for example, around 0.05), and that the overall NPV mortality increases as E increases. For sufficiently large values of E, population crashes result. Some numerical results are shown in table 8-5. For sufficiently high population densities, extremely low initial infection rates produce severe epizootics. An interesting property of the sytem is that an increase in non-NPV mortality can lead to an increase in the overall survival and in the proportion of healthy (that is, not virus-infected) adults, as shown in table 8-6. This is especially pronounced if the increased nonvirus mortality is an abrupt partial kill early in the period, as might result from a pesticide application that was only moderately effective.

One general theoretical comment should be made. The basic mechanisms involved are not highly similar to those assumed for the usual epidemic models. Neither deterministic nor stochastic gypsy moth NPV epizootic models will resemble common epidemic models, and general conclusions of epidemiology reflecting the behavior of such models are not applicable; they could be seriously misleading if

Table 8-5.—*Density-dependent effect of the simulated NPV epizootic*

Eggs at start of generation n	Eggs produced
20,000	106,000
40,000	185,000
60,000	192,000
80,000	134,000
100,000	80,000
120,000	52,000
140,000	41,000
160,000	37,000

Table 8-6.—*Effect of nonvirus mortality*

Initial eggs (E)	Larval mortality factor (R_L)	Pupal mortality factor (R_p)	Eggs produced
80,000	0.016	0.008	139,000
80,000	.020	.010	142,000
80,000	.025	.0125	144,000
80,000	.030	.015	147,000
80,000	.040	.020	148.000
80,000	.050	.025	143,000
80,000	.060	.030	134,000

applied without reverification. For example, no sharp threshold phenomenon will occur, because insects are removed from the susceptible category by pupation.

The simple model can be made more realistic in a variety of ways. One way is to increase the number of compartments and to distinguish sexes. A model was programmed that used separate compartments for male and female insects of each normal stage, compartments for mortality not caused by NPV associated with these, and two NPV-infected compartments associated with the stage/sex compartments, as well as NPV-killed compartments. The model used a system of 130 differential equations. The qualitative behavior of numerical solutions for the expanded system was found to be similar, in general, to that of the simple model written out above. In particular, it was found that an increase in non-NPV mortality, other things being equal, could lead to an increase in overall survival by inhibiting the NPV epizootic. The expanded model still showed some unsatisfactory properties, however, in that significant numbers of adults appeared too soon, and significant NPV mortality occurred too soon. Some bioassay simulations were then developed and discussed with researchers experienced with the disease, to determine how sharp the distribution over time of NPV mortality should be to appear plausible. It was found that approximately 40 compartments, corresponding to successive virus infection stage classes, would be required. For internal compatibility, the insect aging model would then have to operate with age class compartments corresponding to 6-hour (1/4-day) increments. The number of differential equations

needed for such a model approaches 10,000. The size of the system could be controlled somewhat by compromising the sharpness of the time distributions, but other properties of the system suggested that this might not be advisable. Some difficulties were encountered in obtaining numerical solutions even for very simple models, and these became severe with the system of order 130. Apparently the systems are stiff (Gear 1971), so that rapid numerical solution techniques, such as the various elaborations of the fundamental Runge-Kutta method, are unstable and do not converge satisfactorily in computer implementations. It is emphasized that it is not the mathematical system of differential equations that is unstable. The instability is a property of the methods of numerical solution. Methods that produce satisfactory numerical solutions are available, but they are expensive to use.

Current work on functional models is based on the use of finite difference equations with 1-day increments. There is no doubt that instability would appear if a sequence of systems with increments approaching zero was examined. However, use of such a sequence does not seem to be particularly desirable, since it implies a temporal homogeneity that is not very plausible for the real biological system. The use of difference-differential equations was considered and rejected. The reason for the rejection was that the step size in numerical integration of these models should be left free. Otherwise, because rapid changes sometimes occur, the step size must be made very small, and thus storage requirements and computing time become excessive. If the step size is left free, however, the system must be replicated at least several times, corresponding to several delay intervals, and storage requirements and computing time would still be excessive.

The general structure of the functional model in use at the time of writing is indicated in figure 8-8. Because this structure is complex and is also in a state of rapid development, no attempt to discuss completely the details of the model will be made here.

As would be expected, many details are uncertain and not supported by data. The gross features of solutions of the simpler models are preserved in this model, although some are less pronounced. Namely, the intensity of the epizootic is strongly density dependent and can develop from very low initial infection levels for a wide range of model parameter selections. A fairly wide range of generation trend patterns can be obtained, with fairly plausible shapes, showing a high degree of density dependence and severe crashes at high densities.

Ricker (1954) discusses some features of population dynamics most easily seen with the aid of reproduction curves (Ricker's terminology). These are, for gypsy moths, plots of population density in generation $n+1$ against population density in generation n. In particular, he points out that population fluctuations and the stability of an equilibrium point are characterized by the shapes of the reproduction curves and gives examples which lead to various possible cases. The curves produced by the model have approximately the qualitative appearance of Ricker's examples and, for most parameter values tried, resemble the example which produces extreme oscillations.

Several other interesting features also appear in the numerical solutions. One is that if the larval growth equations are structured to produce approximately exponential growth against time, then the overall consumption patterns seem to require a decreasing ratio of leaf area ingested per unit insect weight, as the insect ages, to obtain a satisfactory pattern of overall defoliation. Otherwise there seems to be too little early defoliation, as compared to time patterns of defoliation reported from the field. The solutions are very sensitive to variations of relationships between the distribution over time of bud burst and the distribution over time of insect hatch. At the time of writing, this sensitivity in the model is thought to reflect a biologically real effect; the degree of sensitivity suggested by the numerical solutions may be too high, however.
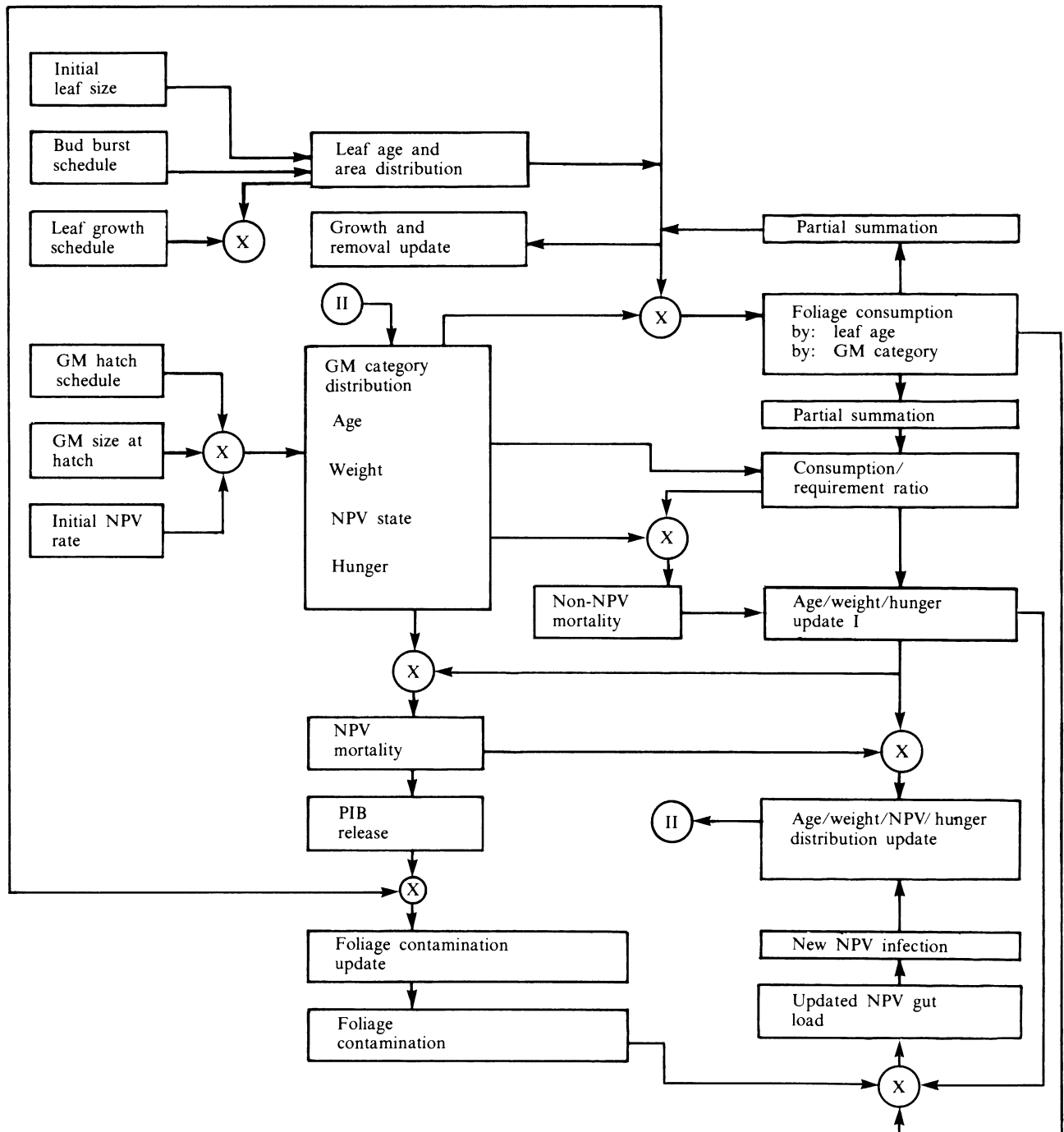
Figure 8-8.—*Functional model of gypsy moth simulation.*

To summarize, the results indicate sensitivity of potential operational importance to the following complexes of possible factors:

- Phenologies of foliage and insect development, especially hatch and bud burst distributions.
- Insect feeding behavior, nutrition, and growth.
- Dissemination, distribution, and infectivity of NPV and interactions with the process of digestion and excretion (presumably this is important for other disease organisms as well).
- Variation, if any, of disease virulence with insect stage, especially the pupal and adult stages.
- Vertical (transgeneration) disease transmission rates and mechanisms.

Several extensions of the functional model outlined in figure 8–8 are under consideration. Selection and incorporation of extensions depend upon data availability, programming and computational difficulties anticipated, and the possible operational implications of the numerical results. Items thought to be of most importance at the time of writing are:

- Weather and climatological effects on phenologies, especially hatch and bud burst.
- Effects of other disease organisms.
- Effects of variations in foliage quality.
- Effects of dispersal.
- Effects of predators and parasitoids.
- Effects of sex-related differences, such as larval development rates and numbers of molts.

Summary

The systems planning and design work for the comprehensive gypsy moth management system is proceeding from the top down, in most respects. This process has the advantage that the major, essential system functions and criteria are identified, and appropriate assignments of responsibility are worked out, in the early stages of the overall task. The activities, interactions, and various kinds of resources needed to perform the major functions are then worked out in increasing greater detail, until the design is completed.

The CGMPMS as it is currently conceived is organized into three levels. The major functions are assigned generally among these levels as follows:

- Level I: Operations planning, logistics, and execution at the local levels of pest surveillance; surveys for environmental considerations, public communications, and intervention; and localized planning and design.
- Level II: Operations planning, logistics, execution and coordination of pest surveillance, surveys for environmental considerations, public considerations, and intervention, especially regional aspects; evaluation and information management, training, state and regional planning and design; and refined resource allocation.
- Level III: Overall policy, resource acquisition and higher level allocation, R&D priorities, long-range planning and design; evaluation and information management; public communications; and systemwide coordination and review.
- Mixed levels: Research and development; some aspects of planning and design; and some activities with containment objectives.

Implementation plans are currently being developed. These include plans for securing approval from the concerned Federal and State agencies. The overall plan involves an evolutionary transition of the existing National Gypsy Moth Advisory Council into a national board at level III, eventually with a small executive staff not within any Federal or State agency. The Gypsy Moth Planning Task Force would evolve into an ongoing planning and design unit of the CGMPMS. The CGMPMS is currently viewed as an organization with very few permanent, full-time members, but many people moving in and out, as the immediate tasks or problems require. Federal and State personnel with pest management responsibilities will continue to be involved in the formulation and implementation of pest management decisions and strategies within the CGMPMS framework, and they will have the means for adjusting the framework as needed. It is expected that the overall system will be operating by the end of 1979; some critical portions will be operating by the end of 1978.

The field activities, primarily carried out at level I, will change slowly in the immediate future, while large-scale operational testing and evaluation of possible improvements in sampling, survey, and forecasting are conducted. The techniques that will be emphasized are egg-mass survey and evaluation, pheromone trap design and sampling schemes using the traps, stand susceptibility and vulnerability rating methods, including tree vigor assessment, and defoliation forecasting. The test and evaluation will probably require three gypsy moth seasons at least.

The techniques of intervention—the tactics of pest management—are discussed in detail elsewhere. Some refinements in the selection and use of agents as insecticides can be anticipated as field experience accumulates, but the details and time frames cannot be specified at this point. Attempts to obtain operationally important responses to manipulation or management of predator and parasite populations have not yet been clearly successful; the intensive work on *Blepharipa pratensis* is continuing, and some critical evaluation data are expected from the 1978 field season. The data currently available allow neither a clear evaluation of the effect of the overall parasite complex nor, most particularly, any prediction of the possible effects of attempts at large-scale management or manipulation. No useful response to small-scale efforts has been demonstrated. Neither of the two major approaches to interference with mating success (confusion using pheromones and sterile-male release) is expected to be ready for routine operational implementation within the next two or three seasons.

Improvement in intervention tactics might be obtained in two general ways. Research and development into application technology could lead to significant improvements in efficiency, especially for the more sensitive biologicals. The goal of work in this area is to reduce losses of the active ingredient. Such losses may arise from many factors, such as droplet evaporation, drift, deposit on inappropriate surfaces such as litter, degradation by ultraviolet radiation, and removal from foliage by rain. Improvements might also be obtained through seeking a closer match between the amount of material used in a particular case and the amount of material actually needed to meet the specific pest management objective. Use of agent combinations might also improve efficiency, through exploitation of synergistic interaction or density-dependent effects or both. Operational considerations are extremely important for the more complex possibilities, and it is most appropriate for these to be addressed by the GMPTF or its successor.

It appears that successful implementation of more subtle integrated pest management tactics may require better fundamental information and more refined data than are currently available. Campbell and Sloan have outlined a complex of tactics at a qualitative level and made some recommendations concerning their use (Campbell and Sloan 1978*b*). Their recommendations are very incomplete, however, and it is not clear in some cases how to complete the specifications. As a particular example, it is very unclear how to seed outbreak foci with parasites with any expectation of useful results; see Parasite Augmentation in chapter 6.1 for a discussion of problems that have been encountered.

Chapters 4 and 5 include discussions of a number of factors believed to be important in the population dynamics of the gypsy moth and the impact of outbreaks. For example, there is no doubt that weather patterns affect the gypsy moth directly and also indirectly, by affecting the complex of natural enemies and the host trees, and possibly foliage quality as well. The quantitative result of the interactions, for particular weather patterns, is generally not known, however. Determination of the importance of some factors, such as the role of dispersal from outbreak foci in the development of regional outbreaks, may require very large-scale operations. The comprehensive gypsy moth pest management system incorporates the capability of blending research, field test, and operations into a coordinated approach to the solution of such problems.

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Summary

9

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Overview

It is difficult to summarize the achievements of the Expanded Gypsy Moth Program because many benefits and spinoffs from this cooperative effort will be realized in the months and years ahead; this compendium can only elucidate many accomplishments and some disappointments that have accrued during this period of intensive research and development. The nine goals listed in chapter 1 were met in whole or in part because of the dedicated effort and support of many scientists and organizations and because a significant base of knowledge existed at the inception of the program.

The more obvious successes include the registration by EPA of Gypchek, the natural virus of the gypsy moth, and two new chemical pesticides, Dimilin® and Orthene®. The development of an efficient mass-rearing prototype facility greatly benefited other scientists in the program who required quality laboratory-reared insects for laboratory and field tests. Because of rapid development in mass-rearing technology, virus production capabilities were greatly enhanced, and scientists were able to recently initiate an interagency thrust to develop and evaluate the sterile male technique for use against the gypsy moth. Significant progress has been made in measuring and predicting the effects of defoliation on trees and stands, and in understanding the processes through which trees are rendered susceptible to the opportunistic organisms that cause their demise.

Although changing trends in gypsy moth populations cannot be accurately forecast, at least two additional factors have been identified—invertebrate predation and foliage quality, which may play important roles in the population dynamics of the insect. Understanding these processes will enable the development of more realistic models in future years.

Despite extensive laboratory and field testing, disparlure, the synthetic sex attractant, is not yet registered for suppression of sparse gypsy moth populations; however, its limitations for this purpose have been defined, resulting in the development of improved formulations and new approaches to use this method in an integrated program to manage the insect. The evaluation, synthesis, and production of the highly purified (+) enantiomer of disparlure by program scientists represent notable achievements that had immediate application. This new, more potent material has been incorporated into the APHIS male moth trapping program, which is by far the most sensitive system for detecting sparse gypsy moth infestations.

Despite the efforts of scientists here and abroad, no known establishment of new parasites in this country resulted from the intensive foreign exploration and introduction program conducted during the past 5 years. Attempts to improve the performance of select native species through augmentative releases met with only mixed success. However, procedures were developed for rearing, evaluating, and improving the performance of parasites that should be invaluable to current and future research studies.

The Current Situation

The severity of the gypsy moth problem has not subsided. In 1975, a substantial reduction occurred in the total defoliation for the region (186,000 ha), and many gypsy moth followers were optimistic that the worst had passed. Unexpectedly, total defoliation doubled in 1976, although declines were recorded in Connecticut and New Jersey. The area of defoliation doubled again in 1977 (to 0.64 million ha), with 80 percent of the total occurring in the vast, susceptible oak forests of Pennsylvania. In 1978, a threefold decrease in defoliation occurred in Pennsylvania; however, this was more than offset by fivefold increases in the States of New York and New Jersey that resulted in a total of 0.52 million ha for the region. The massive defoliated areas and adjacent infested lands that now occur throughout the East pose additional problems because their existence increases the probability that life stages will be accidentally transported to uninfested forests both within and beyond the currently regulated area.

The Future

The ultimate goal of the expanded program—to develop an integrated pest management system to

729

cope with the total gypsy moth problem—is approaching reality. For the first time in years, State and Federal agencies are coordinating their efforts and striving to standardize procedures to detect and monitor populations, measure effects, and evaluate various treatment strategies. This kind of total effort is essential if we are to cope with what is truly a regional problem.

Prior to completion of the Expanded Gypsy Moth Program, it was recognized that a strong continuing research and development effort was necessary to maintain the momentum generated through the program and to complete the promising research that was underway. The Forest Service, Science and Education Administration—Agricultural Research, and Animal and Plant Health Inspection Service responded to this need by funding strong base research programs in fiscal year 1979. Because of this commitment, it is anticipated that many additional benefits will be realized during the next 2 years.

Emphasis has been directed toward improving the performance of microbial formulations and aerial spray technology, integrating various control strategies to reduce populations, evaluating new procedures for using disparlure in sparse populations, evaluating the sterile male technique, improving forecasting models to assist in decisionmaking, and implementing the comprehensive pest management system. Pilot projects have been initiated to demonstrate the capabilities of the virus production prototype facility and to evaluate procedures developed during the program to predict tree mortality and to rate forest stand susceptibility and vulnerability.

It is encouraging and satisfying to those who have been involved in the program that many of the tools and procedures that were developed for survey and detection, evaluation, and control are being incorporated into 1979 State/Federal action programs to suppress, contain, or eliminate gypsy moth infestations.

Appendix A: Program
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Appendix B
Expanded Gypsy Moth
Program Cooperators, 1975–78

Agricultural Research Service (now Science and
  Education Administration)
Animal and Plant Health Inspection Service
Atomic Energy Commission
Bio-Serv., Inc.
Boyce-Thompson Institute
Calspan Corporation
Cape Cod Community College
Commonwealth Institute of Biological Control,
  Commonwealth of Britain
Cooperative State Research Service
Data Courier, Inc.
Electro-Nucleonics Laboratories
Energy Research & Development Administration
Environmental Research Assoc. Laboratories, Inc.
Essex Marine Laboratories
Forest Service
Ketron, Inc.
Library of Congress
Litton Bionetics, Inc.
Maryland Department of Agriculture

Massachusetts Department of Natural Resources
Michigan State University
New Jersey Department of Agriculture
Ohio State University
Pennsylvania Department of Environmental
  Resources
Pennsylvania State University
Rutgers, the State University of New Jersey
State University of New York
University of Connecticut
University of Delaware
University of Maine
University of Massachusetts
University of Michigan
University of New Hampshire
University of Rhode Island
University of Tokyo
University of Wisconsin
Virginia Polytechnic Institute and State University
Yale University

Index

Abiotic factors affecting populations, 22, 28
Accidental introduction to U.S., 1
Acephate, 436, 437, 438, 443
  Degradation of, 436
  Desirability of, 437
  Efficacy evaluations, 437
  Half-life of, 436
  Mode of action of, 436
  Persistence of, 436
  Residue percolation studies, 436
  Toxicology of, 436
Acer rubrum, See Red maple
Acer saccharum, See Sugar maple
Actia jocularis, 345
Actograph, 677
Adaptations in species, 18
Adaptations to bimodal life, 79
Adhesives used in traps, 543, 544
Adults, chemical communication between, 31–32
  See also pheromones
Advecting Gaussian puff dispersion, 198
  Basic model equation for, 174
  Uncertainties with model, 175
Advisory Council, National gypsy moth, 4
Aerial applications, 3, 26, 408, 424, 445, 508–512, 516, 716, 730
  Banning of, 424
Aerial observation of defoliation, *See* Defoliation
Aerial photography for recording defoliation, 41
Aerial sketch mapping, photometric measures regarded as, 40
Aerial trail in mating behavior, 572
Aeronaut spiders, 200
Aesthetic impacts of population, 31
Agar in diets, 611, 643, 644
Age-interval survival rate, 70
Agelaius phoeniceus, See Red-winged blackbird
Agents, biotic, 26, 86
Agonum sp., 110
Agreements, Project-4 Cooperative, 355
Agria affinis, 306, 317
Agricultural Research Service, 5, 6
  Asian Parasite Laboratory, Japan, 303, 340
  Basic Biology and Attractants Laboratory, 677
  Beneficial Insects Research Laboratory, 303, 312, 321, 351
  European Parasite Laboratory, France, 303, 324–340, 341
  Exploration programs' objectives, 309

Agricultural Research Service (Cont.)
  Foreign exploration by, 303
  Overseas studies of, 304
  Quarantine facility of, 311, 412
  Quarantine processing, and, 353
  Systematic Entomology Laboratory, 304, 345
  U.S.S.R., exchange agreement with, 304
Agriculture, U.S. Department of, 1, 5, 32
Agrilus bilineatus, See Two-lined chestnut borer
Air flow and filters for mass-rearing, 621, 622
Air-conditioning system in mass-rearing, 623
Air sampling in mass-rearing, 625
Airborne dispersal, 10, 161
Airflow and dispersion, 189
Akis bacarozzo, 318, 354
Alcohols in oak leaves, 221
Aldrin, 459
Alkaline protease activity and NPV detection, 514
Allelochemics, 657
Alternaria conidiospores, terminal velocity of, 175
American toad (*Bufo americanus*), 109
Amino acids, 221
  Black oaks, in, 223
Amphibian predators, 98, 105
Anastatus bifasciatus, 343
Anastatus disparis, 20, 87, 89, 91, 95, 203, 306, 310, 312, 316, 326, 327, 335, 338, 344, 348, 349, 355, 398, 412
  Establishment of, 348
  Release in Spain, 405
Anastatus japonicus, 342, 343, 355
Anastatus ?kashmirensis, 306, 324, 354, 398–399, 413
Anemomenotaxis, 575, 577, 583
Anemometer bivane used in dispersion studies, 184
Animal and Plant Health Inspection Service (APHIS), 6, 199, 355
  Equipment for mass-rearing at, 624–626
  Mass-rearing facility, and the, 631
  Methods Development Center, Massachusetts, 321, 353
  NPV production, and, 462, 636
  Pesticides, and, 425, 428
  Trapping program of, 729
Anthrenus verbasci, 307, 318, 320
Antifeeding extracts, 657, 665

Antifeeding sesquiterpene lactones, 662, 663
Antigen, group in polyhedrin, 501
Antimicrobial agents
  Diet contamination, against, 612
  List of, 613
Antisera, homologous, 500
Ants, 110, 111, 119
  See also Black carpenter ant
Apanteles, 344
  Alternate hosts for, 314
Apanteles lacteicolor, 305, 317, 320, 335, 339, 365
Apanteles liparidis, 87, 95, 301, 305, 310, 314, 317, 322, 328, 329, 330, 331, 335, 338, 340, 350, 352, 357, 372, 374
  Alternate hosts of, 375
  B. thuringiensis applied with, 409
  Colonization of, 366
  Inundative release of, 407
  Notes on, 366
  Overwintering of, 366
Apanteles melanoscelus, 20, 87, 89, 90, 91, 92, 93, 94, 95, 128, 132, 142, 144, 203, 305, 309, 310, 314, 317, 328, 329, 330, 331, 332, 334, 335, 338, 341, 344, 348, 357, 374, 399–402
  Biological characteristics of, 410
  Biology of, 399–400
  B. thuringiensis applications, and, 451
  Establishment of, 349, 350
  Host/parasite synchronization, and, 400
  Host-selection by, 401
  Hyperparasitism affecting, 400
  Inundative release of, 405, 412
  NPV, and, 478, 482
  Pathogen applied with, 40, 408
  Silk, response to, 401
  Virus applied with, 410–412
Apanteles ocneriae, 317, 367
Apanteles porthetriae, 87, 95, 305, 310, 314, 317, 320, 328, 329, 331, 335, 338, 350, 352, 357, 367, 374
  Alternate host requirement, 340
  Release of, 321, 407
Apanteles praepotens, 317
Apanteles ?ruidus, 367–368
Apanteles solitarius, 314, 315
Apanteles "vitripennis," 310, 311
Apodemus flavicollis, See Old World wood mouse
Apodemus sylvaticus, See Old World wood mouse
Appetitive behavior in the male, 575–576